PROMOTION OF FUNDAMENTAL RESEARCH

IN recent reports, the National Science Foundation has consistently urged the strengthening of fundamental research, support of which, it has urged, is the key to the progress in science upon which technology depends. It has been widely recognized that the proportion of effort devoted to such research in the United States is inadequate, and the 488 million dollars allocated for that purpose in 1959 or the 494 million dollars in 1960 only represent some 6–7 per cent of the total allocation for research and development. Between 1956 and 1959, it is true, expenditure on fundamental research increased by 143 per cent, and now constitutes almost one third of the total research effort in the United States compared with about one fourth in 1956.

Dr. W. R. Brode, Science Adviser to the Secretary of State, in his presidential address to the American Association for the Advancement of Science on December 28, 1959, remarked on the recent increase in the support of fundamental research and the predicted doubling of such research during the next decade. This, he said, gives a high priority to questions of organization. It is of increasing importance to determine the most appropriate means of arranging for financial support, so as to avoid overlapping and ensure continuity; equally, the determination of the right priorities, the selection of programmes and decisions as to their relative position, and the distribution of responsibility for their execution. Dr. Brode is obviously deeply concerned as to the way future policy in science is to be determined and executed in the American scene. Even with the vast resources at the disposal of the United States, neither man-power nor material resources are equal to all demands: selection is inevitable, and Dr. Brode is not satisfied that recent changes made by the President, such as the appointment of a Science Adviser and of a Science Advisory Committee, as well as of a Federal Council for Science and Technology, provide all that is required.

Dr. Brode is obviously watching with interest developments in Britain which may follow on Lord Hailsham's appointment as Minister for Science, and particularly the use which may be made of the Advisory Council for Scientific Policy, though he fully appreciates the wide difference between conditions in Great Britain and the United States. It is interesting, nevertheless, in view of the criticism already expressed in Parliament about the arrangements for representation of the Minister for Science in the House of Commons, to find that Dr. Brode is critical of the refusal of the chairman of the newly created Federal Council for Science and Technology to appear before a Congress Committee inquiring into the possible establishment of a Department of Science. The request was refused on the ground that the chairman was a privileged member of the President's staff. Dr. Brode suggests that while the President should continue to have a strong science adviser, it would be better to separate science co-ordination from the President's own office, so that its head could speak before Congress as well as report the findings and recommendations of all his own agencies to the President.

Dr. Brode has put his finger on a vital matter, of which Lord Hailsham has already shown himself to be fully cognizant. It is impossible to take too great care to ensure that what is involved in scientific policy is fully understood at all levels in the organization of government, both in the legislature and in the administration. While he is clearly not fully satisfied that, in the United States, a Department of Science would be an effective means of interpretation and formulation of policy, he firmly urges the establishment of a commission or study group to consider the organization of science and scientific policy in academic, industrial and government fields. Particularly this should examine the re-grouping of the Government's scientific agencies and activities, either under a Department of Science with a National Science Council, a National Science Institute or some other co-ordinated structure. Effective co-ordination would be required between such a re-grouping and those scientific agencies which remained separate, so as to ensure an efficient and comprehensive national science programme in the United States.

With this Dr. Brode suggests that there should be considered a re-alignment of the methods of distributing responsibility for support of fundamental research in educational institutions, possibly through the administration of university grants mainly by a department concerned with fundamental research, rather than by agencies concerned with application. He also suggests some separation of government-sponsored major research institutions from the educational and industrial system, especially of those institutions essentially concerned with applied science, and finally that scientists in government service should be linked with those in the academic field and in industry on a National Science Council. Primarily, he is concerned to see that creative thought, mature judgment and experience are brought to bear on these difficult problems of the formulation of scientific policy and the co-ordination of activities, and also on the attack on scientific and technical problems themselves.

Dr. Brode is undoubtedly right to maintain that thoughtful and penetrating analysis of the problem must precede any decision to change existing institutions and procedures; and while he is prepared to support drastic action, it is only on the basis of full inquiry. That is known to be Lord Hailsham's attitude, and it is in this context that the papers presented last May at a symposium on Basic

Research in New York and now published by the American Association for the Advancement of Science* are of especial interest. Although fundamental research is only one element involved in the determination of scientific policy, it is probably the essential factor, and the one in which it is most difficult to determine priorities and to exercise co-ordination without detriment to scientific work.

The symposium, which was held at the Rockefeller Institute under the auspices of the U.S. National Academy of Sciences, the American Association and the Alfred P. Sloan Foundation, sought to examine facts concerning the support of basic research in the United States, the reasons why the nation did not give it adequate and suitable support, and ways in which the situation could be improved. Five specific questions were put to the invited participants. Is not the large support of applied research, and still more particularly the massive present support of development, in unhealthy relation to the meagre support for basic research? Is it not true that industry pays eager lip-service to basic research, but in actual fact does not give adequate support to basic research, either within industry or elsewhere? Has either industry or the Government learned how to protect basic research from the insistent demands of applied research and development? Are not universities so deeply invaded by the demands for solving immediate problems, and by the temptation of income for so doing, that there are too few competent scholars pondering problems simply because it interests them to do so, so that scholars in the universities of the United States may have lost the manœuvring room for their continuing re-analysis of the universe? Has it been effectively accepted that the spirit of basic research is an essential ingredient of the educational process—and that this fact should affect educational procedures at all levels?

None of these questions can be dismissed as irrelevant in a British context, and although the answers which might be given may differ considerably, especially in detail, it is a tribute to the quality of the symposium that the addresses reproduced in this volume should bear so closely on current problems of the organization of the research effort in Britain which the Advisory Council on Scientific Policy must now be considering and which impinge closely on the expansion of universities and of technological education. That might indeed be expected from the eminence of the contributors, which include Dr. J. R. Oppenheimer, Dr. A. T. Waterman, Dr. L. A. DuBridge, Dr. J. R. Killian, Dr. C. H. Greenewalt, Dr. A. V. Astin, Dr. J. B. Fish and Dr. M. A. Tuve, and it is noteworthy that in directing the attention of the symposium at the start to the need for new knowledge, Dr. Oppenheimer was not content merely to urge that getting new knowledge was an ennobling as well as a useful activity. Beyond its character as a human, creative activity, he pointed to the wide field of study which man's institutions, his whole society now present—underlining, as it were, the basic contention in Dr. Brode's address already noted.

Dr. W. O. Baker, discussing the paradox of choice for the research worker, followed a very similar line suggesting that it is time we began to consider what specific skills as well as ideals are needed for the advancement of civilization. If a fraction of the effort that has been given to the acquisition of physical skills were given to the practice on proper levels of mathematical reasoning and exercise, we might prepare that small but necessary group which would interpret, as well as the essential group which would create, conceptual science to come. Dr. Baker quoted Dr. Warren Weaver's remark that "our society has not yet evolved a satisfactory way of fostering creative scholarship" in illustrating the potential contribution of scientific method in developing the appropriate institutions for both fundamental and applied research.

The clearest picture of the actual position of fundamental research in the United States was presented by Dr. A. T. Waterman, who, taking the figures for 1956–57, when some 7 per cent of the 9,000 million dollars expended in the United States on research and development went to basic research, said that of 300,000 full-time engineers and scientists estimated as engaged in research and development 27,000 were on fundamental research, 14,000 being in educational and other non-profit institutions, 9,000 in industry, and 4,000 in the Federal Government. Recognizing the vital importance of the universities in this field and the difficulties arising in regard to finance, Dr. Waterman concluded that the evidence shows that fundamental research requires stronger support at colleges and universities; provision for renovation of research laboratories and for up-to-date scientific equipment presents outstanding problems. Clearly, the selection of problems and determination of priorities present very similar problems in the United States and in Britain, and Dr. Waterman seems more confident than Dr. Brode that the new organization in the United States is appropriate to the conditions there. He concedes, however, the importance of the right choice of men, and this is the factor that other participants repeatedly stressed. Dr. Killian remarked that a report recently presented to the new Federal Council for Science and Technology had cited highly trained scientific man-power as constituting the real limit to the development of materials research, and urged the need not simply for more fundamental research but also for more men capable of creative contributions. Dr. C. H. Greenewalt, who claimed that the du Pont de Nemours Company is now spending more than 15 million dollars a year on fundamental research, urged a substantial increase in unrestricted funds for the universities, provided both by industry and government, but based not upon projects but on men—not upon desired objectives but upon creative talent. Dr. M. A. Tuve, of the Carnegie Institution of Washington, commented that

* Symposium on Basic Research. Edited by Dael Wolfle. (Sponsored by the National Academy of Sciences, the American Association for the Advancement of Science, and the Alfred P. Sloan Foundation, and presented at the Rockefeller Institute, May 14–16, 1959, New York City.) Pp. xx+308. (Washington, D.C.: American Association for the Advancement of Science; London: Bailey Bros. and Swinfen, Ltd., 1959.) 27s.

the United States has not had much success, as a nation, in encouraging quietly creative scholarship and intensely personal activity in research; and he suggested that the support of fundamental research is the support of ideas. This always means support of a creative investigator, and he proposed that public funds should be used to pay the salary for the remainder of his working life of a gifted research worker, who had clearly established by the age of thirty or thirty-five his creative ability.

Dr. Tuve visualized the creation in one decade of a body of some 500 or 600 outstanding investigators by the allocation of perhaps 40–60 million dollars a year in creating research professors or scholars of this type; and although in summarizing the symposium, Dr. D. Wolfle recognized that there are both administrative and financial difficulties, he pointed out that there are already precedents in the research professors at universities and the permanent staff of private research institutions. The participants of the symposium, in fact, recommended wider use of research professorships or similar posts as a means of furthering fundamental research.

The other recommendation which came from the symposium was that universities must always be expected to carry the largest responsibility for basic research, and that accordingly they should be provided with larger unrestricted funds. There was also repeated insistence that a larger proportion of the present Federal research and development budget should be available for the universities on an unrestricted basis. Only with a sufficiency of such funds can the universities adequately discharge their continuing responsibilities for teaching and creative scholarship, and maintain a reasonable balance between teaching and research and among the various fields of scholarship. It was equally plain that those present entertained the same distrust of project grants as universities in Britain have of sponsored research and earmarked grants. The vital factor is to free creative scholarship from whatever limits its efforts. It is essential to concentrate on intellectual quality, and while the importance of freedom to investigate and to publish was generally recognized, there was less disposition to concede that the difference between State-owned and privately owned institutions is always as great as is commonly maintained. Dr. DuBridge, in fact, was deeply sceptical of scientists as administrators, particularly in government institutions and when controlling the activities of other scientists through the administration of research grants.

This is not to challenge the freedom of the scientist to decide the conduct of his own research or the choice of his programme: it is rather to challenge the validity of attempting to apply cost-accounting practices too closely, and to stress the need to maintain the appropriate intellectual environment and the conditions of service under which basic research is most effectively pursued. That, as Dr. Fish pointed out, involves a certain freedom in the choice of problems, subject to the criterion of relevance, as well as freedom to carry a project to the point of demonstrating the merit of an idea and then to drop the study and follow the next good idea. These are not, however, inherent rights of individuals, and Dr. Tuve made it clear that the Carnegie Institution of Washington interprets the support of ideas always as the support of a man. Dr. DuBridge saw the essential problem as that of attracting sufficient of the nation's ablest minds to the universities and giving them full encouragement and support in pursuing whatever lines of inquiry interested them.

Admittedly that is a difficult challenge to meet, especially with the mounting costs of equipment and assistance in many fields of inquiry, and even though, in comparative costs, expenditure on academic fundamental research may not, in the United States, be more than twice what it was before the War. Thus the expenditure of fully 800 million dollars, which Mr. R. E. Wilson is inclined to regard as the current total expenditure on basic research in the United States, may be inadequate; and the determination of priorities and the establishment and maintenance of appropriate conditions are unlikely ever to be other than major pre-occupations in the United States as in Britain. The symposium threw up few, if any, novel ideas; but it demonstrated the way in which fundamental and applied research are interlocked and are also related to education and to professional training. The universities and the independent research institutions as well as industry and government institutions have their contribution to make, but in determining the structure in which all these activities are most appropriately fostered and co-ordinated the first consideration should be the promotion of excellence, the encouragement of able investigators and their sustained and adequate support with finance, equipment and assistance.

GALILEO AND EULER AS POPULAR EXPOSITORS

Discorsi e Dimostrazioni Matematiche Intorno a Due Nuove Scienze
Di Galileo Galilei. A Cura di Adriano Carugo e Ludovico Geymonat. (Classici della Scienza, 1.) Pp. xxv+887. (Torino: Paolo Boringhieri, 1958.) 7,000 lire.

Lettere a Una Principessa Tedesca
Di Leonhard Euler. A Cura di Gianfranco Cantelli. (Classici della Scienza, 2.) Pp. xxvi+960. (Torino: Paolo Boringhieri, 1958.) 8,000 lire.

THESE two volumes provide, among many other things, an excellent opportunity for comparing Galileo and Euler as popular expositors, albeit at greatly different levels. Galileo's "Discorsi" first appeared in 1638; published at Leyden, in order to evade the ban imposed by the Inquisition, they incorporate material on which the author had been meditating for a long time, and which is so varied that neither Galileo nor any of his friends succeeded in finding a suitable title for it. Thus the first of the four discussions into which the main portion of the

book is divided actually begins with one of the principal themes—the strength of materials—but soon passes to an examination of the concept of continuity and thence to an essay on transfinite numbers.

The dialogue form in which the book is cast lends itself to, and indeed encourages, this sort of discursiveness. Galileo has distributed his exposition and commentary among three speakers: two of these, Salviati and Sagredo, are former pupils, while the third, Simplicio, is an imaginary personage. It is Salviati's function to enunciate and expound; Simplicio, on the other hand, represents the opposition; and Sagredo, an intelligent layman, not only provides the pretext for a further explanation, in simpler terms, of the author's ideas, but also is used to express concepts for which Galileo did not wish to accept full responsibility.

To obtain an adequate notion of the book's contents, one would have to quote Galileo's own index. The second main theme is the theory of accelerated motion, with application to projectiles; but intertwined with this is a considerable amount of applied mathematics as well as many pioneer results in the integral calculus.

The editors have carried out their task with great skill and devotion; the text, which runs to nearly 600 pages, is accompanied by 300 pages of notes. Moreover, those demonstrations which originally appeared in Latin are here reproduced with parallel translations in Italian. With regard to style, Galileo's own limpid prose furnishes a model which, unfortunately, few of his compatriots have seen fit to adopt.

Euler's "Letters to a German Princess" are a classic of a quite different kind; composed during 1760–62, they were addressed to his pupil, daughter of a Margrave, from whom he was separated by the vicissitudes of war. The letters were first published in French, at St. Petersburg, achieving an immediate and extraordinary success. Translations into most European languages soon followed, and numerous reprints (eight in Paris and six in New York) bore witness to the popularity which the work enjoyed for nearly a century, long after it was largely outmoded: suffice it to remark that the book contains an attack on Leibniz's theory of monads and a defence of Newton as against Descartes—both highly topical matters at the time when Euler wrote.

The reasons for this popularity are very clear. To begin with, the letters give a detailed description of almost the whole of elementary mathematics and physics, including astronomy, as Euler understood them. This part of the work is a masterpiece of exposition; it includes accounts of what were then recent results, such as the first experiments with electricity and the determination of longitude at sea (incidentally, for contributions to this problem Euler himself received a prize of £3,000 from the British Parliament). As a popular scientific encyclopædia the book would be difficult to surpass. But there is obviously another cause for Euler's success: the purely scientific information is coupled with instruction in formal logic, philosophy and religion, designed to combat the spirit of the Enlightenment. In this field Euler was an amateur, and an ill-informed one at that; he made himself ridiculous to his contemporaries.

A perplexing question raised by the "Letters" (234 in all) is the intellectual status of their recipient; one is forced to conclude that she was ignorant of almost everything—there are even lessons in elementary arithmetic—but deemed capable of absorbing anything whatever.

The present version, based on the second French edition, reads very well. Both the Galileo and the Euler are handsome books, and should be in every scientific library.

L. Roth

THE LUNAR SURFACE

Strange World of the Moon

An Enquiry into Lunar Physics. By V. A. Firsoff. Pp. x+226+12 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1959.) 25*s*. net.

PERSISTENT observers of the Moon, many of them amateurs with small instruments and little scientific training, have often reported changes of various kinds in the appearance of the lunar surface; these have tended to be met with scepticism by most professional astronomers; but until the last year or two very few professionals have spent much time on lunar observations, and there is thus a considerable chance that genuine phenomena have been missed. The present book, which attempts to interpret changes seen by the author and other observers in terms of known physical and chemical laws, is timely in view of the indisputable observation by N. A. Kozyrev of a short-lived bright cloud of dust and of C_2 and C_3 gas near Alphonsus in November 1958, and in view of the increased interest in the Moon resulting from the new facilities offered by space rockets.

The author points out that the Moon's orbital plane is inclined at only 5° to the ecliptic, which, together with its large size, suggests that it is a captured planet rather than an off-shoot of the condensing proto-Earth. This leads him to speculate that, as a result of planetary perturbations and tidal action, the Moon may have approached and receded from the Earth several times, each approach having led to distinct mountain-building fault systems in both bodies; but an elementary mathematical treatment given in an appendix shows that rather extreme conditions must be assumed. He rejects the impact theory for the origin of craters in favour of 'lunavoes' (a term coined by J. E. Spurr), which are essentially broken bubbles of gas bursting out from a contracting surface; wrinkle ridges on certain maria are treated as evidence for a viscous liquid filler, rather than dust. Mr. Firsoff attributes ray systems to erosion by running water, fed from large deposits of ice accumulated below the spongy surface rocks, and he suggests that the maria, also, are filled by impure ice. The main changes observed consist of the occasional disappearance and creation of craters, puffs of dark and bright dust or vapour, and local changes in brightness and colour at sunrise, many examples of which have been studied by Firsoff himself with the aid of filters, and which he attributes to the evaporation of snow (or possibly carbon dioxide and other volatile solids). This leads on to a discussion of the lunar climate and atmosphere in which it is shown that, in view of Lyman Spitzer's work on the terrestrial atmosphere and of the presence of appreciable amounts of argon resulting from the decay of potassium, the old idea that all water vapour would have escaped may be quite untrue. Firsoff concludes that the lunar surface, or at least subsurface regions, can

be the seat of extensive climatic and seasonal changes, and even of rudimentary forms of life.

The ideas presented in the book are interesting and highly provocative, and the significance of much of the experimental and theoretical work that has been done is discussed in considerable detail, with helpful technical details in the appendixes. Basically, however, the author's arguments only show that his ideas are barely possible; he has certainly not shown that they are at all likely. In particular, the idea that water is present on the lunar surface is highly implausible, not because of escape, but because of the presence of solar ultra-violet radiation, which is known to dissociate water vapour into hydrogen and oxygen in the upper atmosphere of the Earth; the resulting hydrogen would certainly escape from the Moon. Again, the discussion of craters and mountains fails to do justice to the very strong and detailed case for meteorite impact that has been made by R. B. Baldwin and T. Gold. The value of the book thus lies more in its description of observations and in the questions it provokes than in the answers that it suggests.

B. E. J. Pagel

SECONDARY MODERN SCHOOLS

Your Secondary Modern Schools

An Account of Their Work in the Late 1950's. By J. Vincent Chapman. Pp. 298. (London: The College of Preceptors, 1959.) 12*s*. 6*d*. net.

ALTHOUGH many opinions are given about the encouraging successes or the utter inadequacies of the secondary modern school, these are often coloured by information gathered from one or two areas only. As a result many educationists, including heads and assistants from secondary modern schools themselves, have a distorted picture of the general position in the 3,500 secondary modern schools scattered throughout Great Britain.

Benefiting by the award of the Doreck-Isbister Fellowship by the College of Preceptors, J. V. Chapman has been in touch with about half the total number of schools and here records the information and impressions he has gained from his face-to-face and written inquiries. Chapman sought information under three headings. First, on the amount of time allowed for a certain subject on the school's time-table; secondly, the general syllabus of work; and thirdly, the titles of books being used in the main subjects. From this he has built up a record which, on the whole, is one of good work being done in discouraging circumstances, but which, given more buildings and adequate staffing, could be considerably improved.

The demands on school headmasters and headmistresses are heavy but cheerfully accepted. Her Majesty's inspectors have to be accommodated; parent-teachers' associations have to be stimulated; medical inspections have to be arranged; reports for parents and employers have to be written; school journeys need organizing. These and many extra-mural activities throw considerable strain on the school staff; but, even with school milk and school meals, the masters and mistresses carry on with the main job of providing an all-round general education for pupils of widely varying intellectual levels.

Despite the explosive outbursts of the 'Now-when-I-was-a-boy' school, English is well taught in the 3½ hr. a week which is allotted to it in the average school. The influence of good teaching, however, is soon minimized in homes which culturally know little more than the 'Daily Distress' and Channel 9. About the same time is allowed for mathematics; but here the quality of work done depends quite simply on whether or not the school has managed to capture and retain a competent teacher.

Science teaching is, by-and-large, non-existent. Apart from the odd school with a proud record, the story is one of laboratories waiting for teachers or of teachers, despairing of facilities which take a long time to come, leaving to go elsewhere. It is a sad reflexion that the author of such a book has to fill up a chapter heading with 'Science, including Gardening'. Subjects like history, geography and crafts are well covered although many more schools would teach metalwork if they had the facilities. Physical education is a credit to the schools; a beginning has been made with French and commercial subjects. Domestic science and needlework are given considerable attention in girls' schools, while, in some schools, music is treated with such enthusiasm that "the bugbear of the breaking voice is overcome simply by denying that it exists".

The findings in this factual account of what is going on in secondary modern schools to-day inevitably leads one to question the wisdom of the Crowther Committee in recommending that the school leaving age be raised compulsorily for all children to sixteen. Before this is done the nation should repay the considerable debt to the worthy men and women in secondary modern schools who have managed to make such good bricks with so little straw. Any money available for education should be spent on better buildings, providing adequate staff, and seeing that no longer should some headmasters be able to write that in each year they are allowed to spend a total of 9*s*. 6*d*. on books for each child. Two packets of cigarettes a year!

T. H. Hawkins

"B.D.S." PHYSIOLOGY AND BIOCHEMISTRY

Textbook of Physiology and Biochemistry

By Prof. George H. Bell, Prof. J. Norman Davidson and Prof. Harold Scarborough. Fourth edition. Pp. xi+1065. (Edinburgh and London: E. and S. Livingstone, Ltd., 1959.) 63*s*. net.

THIS text-book, like an old friend, is often referred to by initials rather than by name, and to many students, even dental students, "B.D.S." probably first suggests this book rather than a degree. This new edition comes only three years after the last one—an indication of the popularity of the book—and it is good to see that Spanish and Italian editions are now being published. It would scarcely be expected that many major changes would have been made after so short a time, and the chapter headings are the same as before, except that a chapter on glycolysis and the citric acid cycle replaces one which summarized liver function. Many changes, though, have been made within the chapters to bring the text and bibliographies up to date. The chemical structures are, in many cases, more clearly set out, and there are some new illustrations, including an attractive coloured one of protein chains. This book has

always been notable for its generous provision of illustrations, and students appreciate the way in which so much of the experimental material is presented graphically so that it can be more quickly grasped and more easily remembered. The experimental evidence for the facts is given so far as possible, and this is one of the most valuable qualities of the book.

I was disappointed not to find any discussion of the interesting genetic and biochemical features of abnormal hæmoglobins such as the one associated with sickle cell anæmia, nor anything about the trial of diets low in phenylalanine as a possible means of preventing brain damage in infants suffering from phenylketonuria. In a book used so much by medical students some of the over-generous space given up to structural formulæ of, say, the porphyrins might be better used in some such way. This is not a plea for a larger volume: indeed, the authors are to be congratulated on having made this edition slightly smaller than the one before—something that rarely happens in a new edition. It is greatly to be hoped that they will maintain their enthusiasm for keeping this excellent text-book up to date. If at the same time they find it possible to continue the pruning process, so much the better; future generations of medical, dental and science students will be the more grateful to them.

D. C. Harrison

METEOROLOGY FOR GEOGRAPHERS

Weather and Climate

By Dr. Clarence E. Koeppe and Dr. George C. de Long. Pp. viii+341. (London: McGraw-Hill Publishing Company, Ltd., 1958.) 58*s*.

THE authors of this book are American professors of geography. They state in the preface that it is intended primarily for students of geography needing a more detailed and scientific knowledge of weather and climate than is provided in a first geography course, but lacking the knowledge of physics and mathematics required for a standard course in technical meteorology, and for students interested in a liberal arts training without the intention of becoming professional geographers or meteorologists.

The first eleven chapters set out to give in 144 pages an outline of physical and dynamical meteorology without using any mathematics or assuming any knowledge of physics. This is a very difficult task in which the authors have not succeeded. The sequence of ideas is loose, the essential physical principles are not stated or not clearly brought out, and in places the text would certainly be misleading or puzzling to readers of the type to whom it is directed. What, for example, is to be made of the following sentence about the relation of winds to the contours on the 500 mb. chart, "Winds tend to blow parallel with the contours and thus toward the depressions, which is in accord with the principle, already stated several times, that although air moves down the isobaric slope, it can move at a deflection of as much as 90 degrees to the slope"?

The second part, of 190 pages, on climate is much better though not entirely free from error. The Earth's climates and corresponding types of vegetation are described in fair detail in terms of the Koppen-Geiger classification of them. An attractive feature is the photographs of typical landscapes in the various climatic types. Most illustrations, including cloud forms, are well reproduced, but what would the late G. A. Clarke of Aberdeen, Scotland, have said to the 'acknowledgments' (to the U.S. Weather Bureau) for the two of his photographs in Figs. 11–2 and 11–3 or the quality of reproduction of the first?

The book cannot on the whole be recommended.

G. A. Bull

ANIMAL MYCOSES

Fungal Diseases of Animals

By Dr. G. C. Ainsworth and P. K. C. Austwick. (Review Series No. 6 of the Commonwealth Bureau of Animal Health.) Pp. xii+148+12 plates. (Farnham Royal: Commonwealth Agricultural Bureaux, 1959.) 20*s*.

CLINICAL mycology has never been a favourite subject among pathologists. Lecturers promise to deal with it at the end of the course, time permitting; the hospital bacteriologist unwillingly allows a few mould cultures to lie at the end of the bench until one day, angered by the inexplicable appearance of a green flannel-jacket of *Penicillium* on some precious batch of media, he rises up and indignantly consigns them to the autoclave. Identifying and classifying moulds is a formidable enough task to account for much of the unpopularity of the subject; another cogent factor is the shortage of reference works. Conant's "Manual of Clinical Mycology", published in 1954, relieved the situation as regards fungal diseases of man, and Ainsworth and Austwick have now attempted to codify current knowledge of veterinary mycology. The literature is extremely well covered, and for this reason the book should be of great value to research workers and teachers; undergraduate students (for whom it is said to be partly intended) will probably find much of the detail confusing, and if too little space is devoted to clinical matters for the book to have any great appeal for the practitioner, this is perhaps understandable since neither author is a veterinarian.

It is the contrast between the general ætiological patterns of animal and human mycoses that medical workers and biologists will find most fascinating. The key to the problem seems to be the greater exposure of domestic animals to unhygienic conditions, dirt, dust and spoiled or partly decomposing foodstuffs—in short, to infection with normally saprophytic fungi. Thus aspergillosis of chicks may follow inhalation of dust from mouldy grains or litter; bovine mycotic abortion is associated with the winter feeding of spore-containing hay, mycotic mastitis with the feeding of stale brewers' grains and mould toxicoses with damaged maize and other cereals; in sheep the multiplication of saprophytic Actinomycetes in rain-soaked and rotting wool leads to mycotic dermatitis, and soil or mud contamination of the limbs is a major factor in the ætiology of cattle farcy, a form of nocardiosis recalling in many ways Madura foot in the human subject. This is a theme which might well be elaborated in a later book, and who better than Dr. Ainsworth with his unique knowledge of both medical and veterinary mycology to do so?

J. E. Smith

Beetles of the British Isles

By E. F. Linssen. First Series: The Superfamilies Caraboidea, Palpicornia, Staphylinoidea and Diversicornia. Pp. 300+39 plates. Second Series: The Superfamilies Clavicornia, Heteromera, Lamellicornia, Phytophaga and Rhynchophora, and including the Strepsiptera. Pp. 295+87 plates. (Wayside and Woodland Series). (London and New York: Frederick Warne and Co. Ltd., 1959.) 60*s*. net the set.

THE aim of the "Wayside and Woodland" series is to enable naturalists and others to identify animals and plants in the field, chiefly by comparing their specimens with good-quality figures. In the present work the figures of adults are taken mainly from Fowler (1887–1891) and those of larvæ (as usual) from Schiödte (1861–1883). The latter have been adapted by Mr. A. F. Stuart, who is also responsible for the text-figures and the colouring of the plates. His work, original or adapted, is excellent. Of the 126 plates, 64 are coloured.

Many beetles can only be examined satisfactorily under a microscope, and in this work fine structural characters are given so that closer and more reliable determinations can be made than by using figures alone. There are keys to the families of some major groups. The system of classification adopted is that of Kloet and Hincks (1945). A most useful table is given in both volumes showing the superfamilies of Polyphaga recognized by various authors (1 : 140–141 and 2 : 16–17).

A work of this kind must inevitably take the form of a catalogue of species, with notes, and the author should be able to express himself clearly, concisely and authoritatively. Unfortunately, Mr. Linssen's style is cumbrous and many of his statements are inaccurate or obscure. Particularly deplorable is the large number of misspellings of scientific names which cannot fail to confuse the amateur, especially as genuine obsolete spellings are often given.

R. T. THOMPSON

From Ape to Angel

An Informal History of Social Anthropology. By H. R. Hays. Pp. 461+32 plates. (London: Methuen and Co., Ltd., 1959.) 36*s*. net.

BOOKS to popularize science may take many forms. Very attractive are those where some great man of science takes a recent discovery and translates it into language which can be understood by those whose knowledge of science is limited, but who still have inquiring minds. Equally valuable is the general interpreter, a man who appreciates the interest of the general public in a particular aspect of science at a given time, and distils knowledge from a wide variety of sources and presents it in an attractive and readily assimilable form. Because these works contain little that is wholly new and, for the sake of simpleness, sometimes put considerable strain on veracity, they are often dismissed with contemptuous references to 'pot-boiling'. Hays's popular narrative of man as a social animal, including accounts of his primitive behaviour, could be put in the latter category. Yet here, to match up with the considerable interest in the lives of primitive peoples which has been evoked by television, is a book which may well develop into a best-seller. To describe the extraordinary ethical, religious and marital customs of primitive peoples, Hays provides a short biography of the lives of famous pioneer anthropologists such as Henry Shoolcraft, Westermarck, Sir James Frazer, Franz Boas, Bronislaw Malinowski and others. The result has been the production of an informal history of social anthropology which is attractive and informative and which does the author credit for the assiduous way he has collected his information and put it together. "From Ape to Angel" has deservedly won recommendation from the Book Society.

T. H. HAWKINS

The Thyroid Hormones

By Dr. Rosalind Pitt-Rivers and Jamshed R. Tata. With a Chapter on Diseases of the Thyroid by W. R. Trotter. (International Series of Monographs on Pure and Applied Biology. Division: Biochemistry, Vol. 1.) Pp. xiii+247. (London and New York: Pergamon Press, 1959.) 50*s*. net.

A BOOK by two such authorities on research into the thyroid gland could scarcely fail to be useful. The authors say that their aim is to unfold the story of the thyroid hormones and to discover which hypotheses concerning their biosynthesis, mode of action, and the ætiology of thyroid disorders, have received experimental support. They also have tried to provide research workers in the field of thyroid physiology and biochemistry with as many references to the recent literature as possible.

The unexpected appearance of tri-iodothyronine on the scene in 1952, in which Dr. Pitt-Rivers herself played a leading part, has led to much new thinking about thyroid hormones. We are still without a finally accepted view about the nature of thyroid hormones and their mechanism of action, but the present book sets out as much as can be reasonably concluded at the present time. More information about the actual amounts of the different thyroid hormones found in blood and tissues under physiological conditions might have been expected, but undoubtedly much checking and re-checking must be done before final conclusions are drawn. The authors have clearly succeeded in their formulated aims in writing this book.

F. G. YOUNG

Dana's Manual of Mineralogy

Revised by Prof. Cornelius S. Hurlbut, jun. Seventeenth edition. Pp. xii+609. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 78*s*. net.

JAMES DWIGHT DANA'S classic "System of Mineralogy" has stood the test of time, so also has his "Manual of Mineralogy", which has now reached its seventeenth edition. The first edition of the "Manual" appeared in 1848; since then many revisions and editions have appeared, the last being in 1952. The latest revision was undertaken by Cornelius S. Hurlbut, jun., professor of mineralogy in Harvard University. It introduces the broad use of the crystal-chemical approach, and many of the chapters have been expanded in this light. The chapter on "Crystal Systems" now includes discussions of the stereographic projection, the thirty-two crystal classes, calculation of axial ratios and X-ray crystallography. This new approach enables the properties of minerals to be treated through fundamental structures and chemical consideration. It is hoped that if future revisions are made care will be taken not to resolve the "Manual" into a form indistinguishable from the "System of Mineralogy". It is better that the "Manual" maintains its original purpose, for students, amateur collectors and mining geologists.

INCREASES IN THE LOCAL GAMMA-RAY BACKGROUND DUE TO NUCLEAR BOMB FALL-OUT

By J. VENNART

Radiological Protection Service, Belmont, Sutton, Surrey

IN 1956, prior to the large-scale testing of thermonuclear weapons, Spiers and Griffith[1] and I[2] made measurements of the local gamma-ray background in Leeds and Aberdeen, and in Belmont and London respectively. A noteworthy feature of the measurements was that, due to the low radioactivity of the underlying chalk of the North Downs, the local gamma-ray background to which man is exposed in Belmont was about 60 per cent of that in Leeds. It is now known that this is principally due to the small amount of potassium in chalk (about 20 parts per million compared with 5 per cent in common building materials), giving a correspondingly low concentration of the naturally occurring radioisotope potassium-40. In addition, the contribution from the other principal naturally occurring radioelements, thorium and uranium, is about ten times less than that in common building materials[3].

These facts have had important repercussions with regard to the design of low-background laboratories within the chalk at Belmont, which are used for the measurement of low levels of radioactivity in humans[4] and in food[5]. We are therefore concerned about any increase in the local gamma-ray background of our environment, and accordingly in April 1958, shortly after our low-background laboratory was completed, I initiated regular checks out of doors at the Belmont Hospital and have continued these to the present day. In addition to these measurements I have also on one or two occasions repeated the measurements reported for 1956 inside and outside a dwelling house at Belmont and at other spots in and around the Hospital. The results show that during the period of the measurements, the local gamma-ray background out of doors increased steadily and reached, in April 1959, a maximum which was about twice that in 1956. In recent months there has been a spectacular fall, presumably due to the cessation of nuclear testing, and at the present time the local gamma-ray background out of doors is barely significantly different from that measured in 1956. As regards the local gamma-ray background inside buildings, measurements made on two occasions indicated that the increase varied considerably with the shielding effect of walls but might be in the order 50 per cent of that reported out of doors. I am reporting these measurements now so that a comparison may be made with others reported recently by Peirson and Salmon[6] for various parts of Britain and by Spiers[7] for Leeds, and also so that the magnitude of this increase in relation to man's previous radiation history may be discussed.

The measurements were made, as they were in the early experiments[2], with two counters *G*.10 lead and *G*.10 carbon enclosed in brass cases 1½ in. in diameter and 1·2 mm. thick. The same *G*.10 carbon counter was used for the whole period, including the 1956 measurements, whereas, due to breakages, it was necessary to use four different *G*.10 lead counters. However, these had previously been calibrated against a radium standard and their sensitivities were the same within 2 per cent. All the counters were checked regularly for constancy of response with a weak radium source. The residual counter response to cosmic radiation and counter contamination was determined by shielding experiments out of doors and also within the 9-in. thick steel enclosure used by Spiers at Leeds. The value obtained was approximately 40 counts/min. for either type of counter. As described in the earlier paper, the ratio of the counting rates due to local gamma-rays from the two counters at a given site is a measure of the relative proportions of primary radiation and scattered radiation in the local gamma-ray background. This ratio has remained substantially constant at about 2·1 for all sites throughout the whole series of measurements. It may then be shown[3] that the local gamma-ray dose-rate in m.rads/yr. is given very approximately by (1) counting rate due to local gamma-rays in the *G*.10 lead counter multiplied by 0·9, (2) counting rate due to local gamma-rays in the *G*.10 carbon counter multiplied by 1·9.

Fig. 1 shows the net counting rate out of doors due to local gamma-rays (left-hand ordinate), for the *G*.10 lead counter placed about 3 in. above closely mown grass outside the low-background laboratory. Apart from December 1959, when only two measurements were taken, about forty individual measurements were made each month; for any single measurement the standard deviation of the counting was about 2·5 counts/min. The mean value for any day was found from three to four separate measurements of the background; and the graph shows, for each month, the mean and the standard deviation of the distribution of these daily mean values. An interesting feature of the measurements was the increase in counting rate which followed heavy rain. For example, in August 1958, during a heavy thunderstorm, the counting rate due to local gamma-rays rose to 85 counts/min., but within 4 hr. of the rain ceasing this had fallen to about 42 counts/min. A large fraction of the difference, 43 counts/min., may therefore be attributed to the gamma-emitting decay products of the naturally occurring gas radon which decay with an effective half-period of the order of half an hour. No measurements which were taken during or immediately following heavy rain have been included in the graph.

The graph also shows (right-hand ordinate) the dose-rate in m.rads/yr. using the calibration factor given above. Although these values represent the dose-rate at 3 in. from the ground, other measurements made at a height of 2 ft. gave similar values. This result is in accord with the well-known fact that the local gamma-ray dose-rate is independent of height above the surface of the Earth, provided this does not exceed a few metres[8]. Accordingly, the dose-rate values shown on the graph are, to a close approximation, the local gamma-ray dose-rates to which man has been exposed at the site.

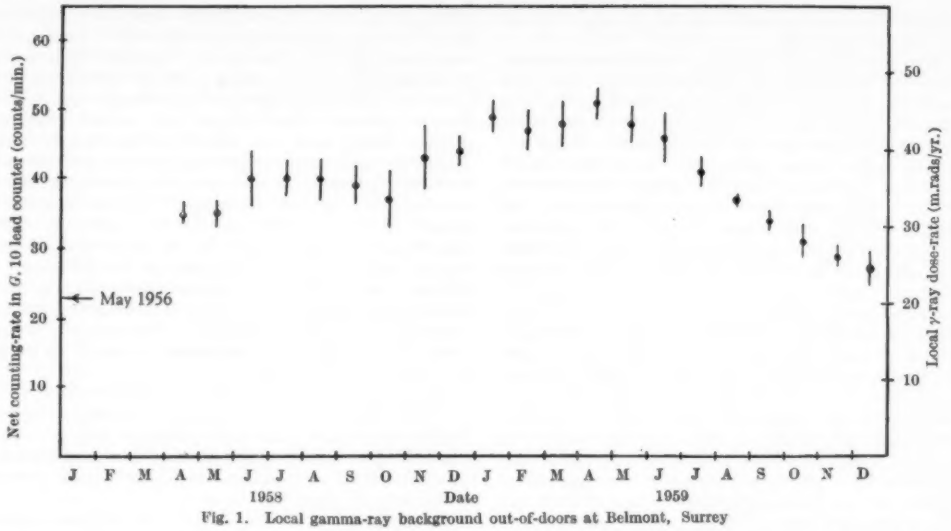


Fig. 1. Local gamma-ray background out-of-doors at Belmont, Surrey

Measurements were also made with the carbon counter on five occasions throughout the series, and the dose-rate so determined agreed with that obtained from the *G*.10 lead counter to better than 5 per cent.

It will be seen from the graph that in April 1958, when the present series of measurements began, the counting rate due to local gamma-rays was 12 counts/min. greater than the value, 23 counts/min., obtained in May 1956 at the same site. Measurements at two other sites, one over a tarmac courtyard within the Belmont Hospital grounds and one over the garden of a house in Belmont, at which measurements had been made in early 1956, showed similar increases in counting rate, namely 15 and 11 counts/min. respectively. It is of interest to note that the increase in counting rate was about the same over closely mown grass, cultivated garden and tarmac. This evidence, coupled with that of Spiers[7], would imply that it may be over-optimistic to use a protection factor of 3, as was suggested in Appendix *M* to the Medical Research Council's report of 1956[9], to take account of fall-out material which is washed into drains or otherwise removed from the topmost layer of the surface of the Earth. From April 1958 the counting rate continued to increase steadily, apart from a fall in October 1958, to a maximum in April 1959 which was more than twice the 1956 value. The corresponding dose-rate at this peak period was about 25 m.rads/yr. more than in 1956. By December 1959 the counting rate due to local gamma-rays was barely significantly different from that in 1956. The dose-rate outside the house in Belmont was also barely significantly higher than in 1956. The effect of the series of tests during 1957–58 and especially in October 1958, as well as the cessation of all tests since that time, is clearly demonstrated.

From spectrometric measurements we have made of the radioactivity in the air during the period, there can be little doubt that the increased counting rate was due principally to gamma-emitting fission products of relatively short half-lives, less than one year, namely zirconium-95 and its daughter niobium-95, together with ruthenium-103 and rhodium-106. The longer lived cæsium-137 would be present to a very much smaller extent due to the much lower fission yield. The variation of counting rate with time shown on the graph is in fact very closely in step with that shown by Peirson and Salmon[6] for the cumulative deposition of fission products at Milford Haven. The maximum increase in the local gamma-ray dose-rate, 25 m.rads/yr., which occurred during April 1959, is the same as that obtained by Spiers during May 1959 over a lawn in Leeds[7]. It is also very close to the value which may be calculated for Belmont, where the mean annual rainfall is about 25 in., on the observed relationship between rainfall and cumulative fallout[6].

Using both counters, the local gamma-ray background was measured indoors during April 1958 in one ground-floor laboratory at the Hospital and at several sites on the ground-floor of the house in Belmont. There was no significant increase above the values obtained in 1956 for the room in the Hospital and the average increase in the house amounted to about 4 m.rads/yr., which was about equal to the standard deviation of counting the difference. The maximum increase in the house was about 8 m.rads/yr., compared with about 10 m.rads/yr. out of doors at the same time. Large variations in the local gamma dose-rate indoors are of course expected due to the different shielding effects of walls and windows. For example, in the Hospital, where no increase in dose-rate was measured, the counter was protected from fission products on the ground outside by brick walls 20 in. thick. It is clear, however, that it might be unduly optimistic to assume a protection factor of 7 in all cases for the shielding effect of buildings, as was suggested in Appendix *M* to the Medical Research Council report[9]. It was unfortunately not convenient to make measurements indoors during April 1959, the peak period for the outdoor measurements, but the presence of fission products indoors at this time was clearly demonstrated by measurements of the gamma-ray spectrum from the refuse from a vacuum cleaner which was being used at the house in Belmont. By November 1959, the local gamma-ray dose-rate in the house was not significantly different from that measured in 1956, demonstrating that any fission products inside the building

had decayed to a negligibly low level, in step with the conditions out of doors.

The significance of an increase in the local gamma-ray dose-rate outside of about 25 m.rads/yr. has been discussed very fully by Spiers[7]. It will be clear that the effective increase in man's radiation status is markedly dependent on the assumptions which are made with regard to the protection factor afforded by wash-off into drains and into the deeper layers of the soil, by the shielding effect of buildings and by the fraction of the day spent out of doors. The measurements reported here would indicate that the protection factors assumed in Appendix *M* to the Medical Research Council's report of 1956 [9] with regard to the first two are possibly over-optimistic. There may also be large numbers of people for whom a period of 2½ hr. out of doors, the value used in the report, may also be optimistically low. In fact the measurements reported here are not inconsistent with the most pessimistic factors suggested by Spiers[7]; namely, that the dose-rate indoors due to fission products might be as much as 50 per cent of that outside, and that the dose-rate outside is roughly independent of the nature of the ground surface. If only 2½ hr. are spent outside each day, it would then follow that, by April 1959, the local gamma-ray dose-rate to which man was exposed, due to fission products in the environment, was about 14 m.rads/yr. greater than in 1956. Assuming that body tissues reduce the gonadal dose to two-thirds of this value[7], the genetically significant increase in the annual dose-rate would be about 9 m.rads. This is about 9 per cent of the total from the natural background radiation, which includes local gamma-radiation, cosmic radiation and radium and potassium-40 in the body[9]. If, therefore, testing recommenced and fallout continued at the same rate as 1957–58, the increase in man's gonadal dose from fission products on the ground might itself be greater than the total from all other man-made sources of radiation with the exception of that received from diagnostic X-rays[9]. It might be worth noting that plants and animals which are not protected by the shielding effect of buildings would receive additional gamma-ray doses about three times greater. They might also receive additional genetic doses from the beta-rays emitted by fission products, whereas the human gonads are protected from this source of radiation by the shielding effect of overlying tissues and clothing. The local gamma-ray background is, however, at the moment barely significantly higher than before large-scale nuclear testing began and, if there are no further tests, we might conclude that the increment of dose over the past three years is of small importance compared with, for example, the difference between the doses of natural background radiation experienced by persons in Leeds[1] and Belmont[2].

There will also be small additional doses to the whole body and the skeleton from internally deposited fission products, principally cæsium-137 and strontium-90 respectively. A report of our measurements of cæsium-137 in humans during 1958 and 1959 will be published shortly.

It is a great pleasure for me to record my appreciation of the work done by Miss S. Terry, who has been responsible for almost all the experimental work; amounting to about 1,000 separate measurements of the local gamma-ray background.

[1] Spiers, F. W., and Griffith, H. D., *Brit. J. Radiol.*, **29**, 175 (1956).
[2] Vennart, J., *Brit. J. Radiol.*, **30**, 55 (1957).
[3] Turner, R. C., *et al.*, *Nature*, **181**, 518 (1958).
[4] Maycock, G., and Vennart, J., *Nature*, **182**, 1545 (1958).
[5] Mayneord, W. V., *et al.*, *Nature*, **182**, 1473 (1958).
[6] Peirson, D. H., and Salmon, L., *Nature*, **184**, 1678 (1959).
[7] Spiers, F. W., *Nature*, **184**, 1680 (1959).
[8] Hultqvist, B., "Studies on Naturally Occurring Ionizing Radiations" (1956). Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, 101 (1958).
[9] "The Hazards to Man of Nuclear and Allied Radiations" (H.M.S.O., 1956).

ANTIBODY PRODUCTION

By Dr. STEPHEN V. BOYDEN*

Tuberculosis Immunization Research Centre, c/o Statens Seruminstitut, Copenhagen

THE mechanism of acquired immunity is as much a mystery to-day as it was sixty years ago. The facts which have come to light since then have provided little basis for choosing between the ever-increasing number of theories of antibody production. Even aspects of Ehrlich's side-chain theory[1,2], which was dropped almost completely for a time as a result of Landsteiner's work, are now being discussed seriously again, especially in view of some recent suggestions on the nature of immunological specificity[3,4].

The starting point for most speculation and experimental work on the mechanism of acquired immunity is, not surprisingly, the production of specific circulating antibodies in mammals. However, the failure of this approach to bring us much nearer to understanding the processes involved suggests that perhaps more consideration should be given to the possible origins of the phenomenon of antibody production in evolution. Some speculation from such a phylogenetic point of view has resulted in the suggestions which are discussed in this article. Although the hypothesis which is formulated does not seem to have been put forward in its entirety before, a large part of it is constructed of elements of the previous theories and ideas of others[1–9].

One of the most impressive features of the immune mechanism in vertebrates is its capacity to distinguish between constituents of the body and components of other organisms. Since the ability to discriminate between 'self' and 'not-self' is a necessary attribute of the phagocytic cells of the simplest multicellular animals, it is reasonable to suspect that this particular characteristic of immunity goes back very far in evolution—to periods long before the phenomenon of antibody production came into existence. In other words, the capacity for self-recognition is the most elemental of all the characteristics of the immune response, and it is therefore all the more remarkable that some of the important antibody theories scarcely consider this aspect of the problem, while in others the explanation for it

* Present address: John Curtin School for Medical Research, Australian National University, Canberra.

represents the most far-fetched and the least satisfactory part of the whole argument.

It is becoming increasingly evident that the specific structure of molecules on cell surfaces in both unicellular and multicellular animals is an extremely important factor in connexion with the normal functioning and interaction of cells. Specific surface affinities or compatibilities are apparently responsible for a wide variety of cytological phenomena which involve adherence of one cell to another. For example, 'fertilizin', which coats the ova of various marine animals, has a specific affinity for the 'antifertilizin' on the surface of the sperm[10], and the re-aggregation of sponge cells which have been scattered mechanically is apparently due to the interaction of specific surface components[11]. It is also highly probable that surface 'antigens', characteristic for each type of cell, play a vital part in the organization of tissue in morphogenesis[12–14].

An interesting example of the importance of specific surface components in Protozoa, particularly in relation to feeding mechanisms, is provided by the case of *Entamoeba hartmonella* in which bacterial 'agglutinins' have been described[15]. If a suspension of this amoeba is mixed with certain flagellated bacteria, the latter can soon be seen 'clumped' on the surfaces of the Protozoa. Afterwards phagocytosis of the bacteria occurs. Apparently the fixation is due to the presence on the surface of the amoeba of molecules which have an affinity for components of the flagella of the bacteria.

It is not unlikely that in simple multicellular organisms in which phagocytes play such an essential part both in nutrition and in defence, similar surface factors may form the basis of the mechanism by which these cells recognize the foreign matter. We can envisage that the phagocytes are, like *Entamoeba hartmonella*, coated with molecules with structures complementary to bacterial components. However, for phagocytes to be effective in the defence of the animal against infection they must have an affinity for a much wider range of micro-organisms than does *Entamoeba hartmonella*. It is therefore necessary to assume the presence on their surfaces of a large number of different molecules of varying steric configurations.

Concerning the synthesis of these molecules, it is possible that the structural variation essential for the wide range of specificities might be achieved by the presence in the cells of a corresponding number of distinct ribonucleic acid templates. An alternative and somewhat more economical possibility is that only a single type of ribonucleic acid template is needed; but that the structure of the protein molecule is such that it is extremely versatile with respect to the number of possible shapes that it can take up as it folds after coming off the template (cf. ref. 6). The final specific affinity of each molecule would thus depend upon the shape taken up during this random folding process.

To account for the ability of the phagocytes to distinguish between 'self' and 'not-self', two possibilities can be considered. First, we can suppose that two types of cell are implicated in the process. One of these cells produces, and liberates into the body fluids, molecules possessing random affinities but all of which have also a second affinity for the surface of the phagocytes. Molecules in which the randomly determined specificity is complementary to self-components are soon 'neutralized' and removed from the body fluids. The others continue to circulate in the fluid until adsorbed by the phagocytes by virtue of their second and constant affinity for the phagocyte surface. Thus the phagocytes become coated with molecules complementary for many different 'determinant groups' other than those occurring on self-components. This idea of molecules with such 'double specificities' is not entirely fanciful, since it is known that sera of immunized mammals sometimes contain antibodies which have an affinity not only for the immunizing antigen but also for the host's own cells. Normal cells which have been treated with sera containing such antibodies are afterwards capable of specifically adsorbing the antigen[16–18].

An alternative possibility would be that the phagocyte, like *Entamoeba hartmonella*, synthesizes its own 'receptor' molecules, but that the production of these ceases before the cell reaches maturity and before it is able to 'phagocytose'. Molecules with an affinity for 'self' would become 'neutralized' early on, so that by the time the phagocyte reaches maturity, only 'not-self' affinities would remain.

To account for so-called 'natural antibodies' in invertebrates we can postulate the production and release into the body fluids of molecules with random specificities but with no special affinity for phagocytes. Molecules with reactive groups for self-components are quickly absorbed out, thus never reaching harmful concentrations. Only molecules with 'not-self' affinities would tend to accumulate in the fluids.

It is suggested, then, that protein molecules of random affinities, especially coating phagocytes, play an essential part in the processes of feeding and microbial defence in the primitive Metazoa. We can now turn to discuss the specific immune response of higher animals, taking the view that its mechanisms are basically similar to, and in fact evolved from, those which are responsible for the ability of invertebrate phagocytic cells to recognize and respond to foreign matter.

The first step in antibody production must almost certainly consist of the uptake of the antigen by some kind of cell, and although absolutely nothing is known of how this comes about, it is not difficult to imagine that the specialized antibody-forming cells are coated, as was postulated for the phagocytes of invertebrates, with 'receptors' for a wide range of foreign molecules (antigens). We may suppose that these receptors are closely related to, if not identical with, γ-globulins. The actual shape and hence specific affinity of each receptor molecule might again be determined solely by the random folding process which follows its release from the ribonucleic acid template.

The usual inability of individuals to respond immunologically to their own proteins can be explained simply by assuming that the antibody-forming cells possess no receptors for self-components. The absence of 'self-receptors' on the cells can be accounted for, as in the case of the invertebrate phagocytes, by supposing either that the 'antigen receptors' are produced elsewhere and so 'neutralized' by the body's own components before they reach the antibody-forming cell, or that they are produced by the immature antibody-forming cell itself, and 'neutralized' before the maturation and development of the full potentialities of the cell. In either case the antibody-forming cells will possess no receptors capable of reacting with any proteins present in the circulation before the antibody-forming system itself

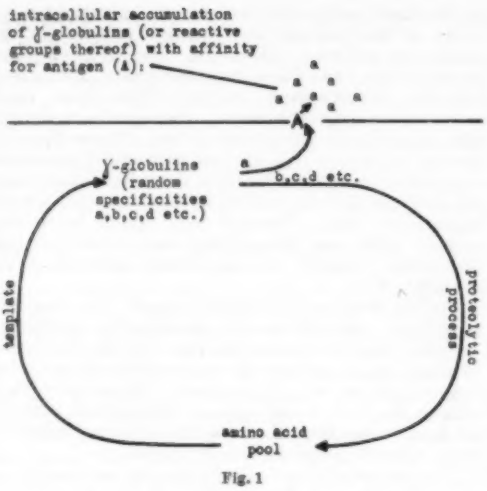


Fig. 1

matures. This, of course, would be expected to apply also to foreign molecules if they happen to be present in the body before maturation of the immune mechanism (for example, at birth) and as long as they persist in high enough concentration to neutralize all the complementary receptors which are produced.

In connexion with the actual production of specific antibodies by the antibody-forming cell after it has adsorbed antigen, we make the following assumptions. In the first place, this specialized type of cell is capable of producing γ-globulin molecules of random specificities at an extremely high rate. Secondly, the cell is capable of breaking down these molecules at about the same rate as they are synthesized. In other words, there is a 'globulin cycle', consisting first of the synthesis from an amino-acid pool of γ-globulins of different specificities, and then of their catabolism by a proteolytic system to the constituent amino-acids.

It is assumed that the antigen, after combining with the 'antigen receptor', enters the cell and that the whole molecule (or determinant groups from it) becomes fixed to some cell component in such a way that it is protected from enzymatic breakdown, rather as has been postulated for the penicillin molecule in penicillinase adaptation in *B. cereus*[19]. The complex, consisting of antigen (or determinant group) and a cell component, is placed within the cell in close proximity to the site of the γ-globulin cycle of synthesis and breakdown. In this situation it interferes with the cycle by drawing aside any molecules which have an affinity for it, passing them on to a storage site elsewhere in the cell (Fig. 1). There is thus a competition between the proteolytic system and the antigen (or determinant group) for all molecules which tend to have an affinity for the antigen (or determinant group). Molecules with no affinity for the antigen will, of course, be broken down as previously.

Thus it is postulated that the presence in the cell of the antigen (or determinant group) is soon followed by the intracellular accumulation of γ-globulin molecules (or perhaps reactive pieces of γ-globulin molecules[20]) possessing an affinity for it. Once this accumulation has occurred, the cell itself must be regarded as 'modified'; perhaps some of the antibody molecules find their way to the cell surface, rendering the cell capable of taking up many more molecules of the same antigen. The consequent increase in uptake of antigen results in further accumulation of antibodies. The metabolic effects of these intracellular changes may be expected to stimulate cell division, so that, in the presence of antigen, 'modified' cells tend to multiply at a greater rate than 'normal' cells of the same series, resulting in the formation of clones of antibody-forming cells. Sooner or later most of the specific globulins, or antibodies, are released into the circulation.

It could be argued, on teleological grounds, that such a process would seem unlikely because it involves an enormous degree of wastage in the synthesis of γ-globulin. However, there are, of course, numerous examples in Nature of apparently wasteful phenomena. Moreover, in this instance there is no actual loss of building material, since the amino-acids are returned to the pool to be used again for further synthesis of γ-globulins, and the 'wastage' applies only to the energy used in the 'globulin cycle'. On the other hand, it is not essential to the main hypothesis to assume complete breakdown of the γ-globulin molecules. One could also suppose, for example, that only the last stage of the process, namely the folding, is reversible and that there exists a pool of γ-globulin molecules under conditions which allow a constant oscillation between the folded and the unfolded state, so that molecules with new affinities are continuously being formed. This possibility comes close to Pauling's theory[6], but differs from it in that it implies a selective rather than an instructive role of the antigen's determinant group.

Since this hypothesis does not require that the antigen modifies, either directly or indirectly, the structure of the γ-globulin molecules synthesized, it qualifies for inclusion in the category of 'elective', rather than 'instructive' antibody theories [21,22]. However, unlike the 'clonal selection theory'[21–25], which is also in the elective group, it does not assume any nuclear or somatic mutation. It does postulate a selection of certain cells, but it is a selection of cells which have become modified as a result of contact with antigen, and not, as in the clonal theory, a selection of genetic variants. (An essential difference between a mutant and the 'modified' cell is, of course, that the former would retain its capacity to be stimulated by antigen even if it continued to multiply for many generations in the absence of the antigen; the modified cell would not.) In this respect the possibilities discussed here are similar to the ideas put forward by Monod[9] and Pappenheimer *et al.*[26], who compare antibody formation with adaptive enzyme production in bacteria. They suggest that the antigen may be captured by a specific 'permease' on the cell surface, and that afterwards more of the same permease is produced so that the capacity of the cell for taking up the specific antigen is greatly increased. The increased uptake of the antigen results in further production of permease and of antibody, and also stimulates cell division.

According to the clonal selection theory, there exist in the mammalian body a vast number of different clones of antibody-forming cells, each producing its own special γ-globulin. When antigen enters the circulation it is thought to attach itself to the cells which have reactive sites corresponding to the determinant groups of the antigen, this resulting in a selective advantage for these particular cells. The

consequent shift in cell population is reflected by an increase in the circulation of γ-globulins of the type produced by the selected cells. It is suggested that cell variants possessing reactive groups for self-components are eliminated either in the embryo[20] or before they reach maturity in the adult[21,22]. Since each cell is supposed to possess reactive sites for only one determinant group (or perhaps a few determinant groups), the elimination of all immature cells with self-reactive sites would mean the destruction of only a small proportion of the total number.

For several reasons the clonal selection theory, in contrast to the hypothesis put forward above, seems to imply that the mechanism by which the antibody-forming system recognizes foreign matter is in principle different from that by which the phagocytes of primitive Metazoa distinguish between 'self' and 'not-self' components. For one thing, the number of phagocytes in these minute animals is relatively very small, so that the chances for variation must be severely limited. Moreover, each phagocyte seems to have an affinity, not for just one determinant group, but for an almost infinite variety of foreign molecules. Considering the large number of reactive sites which this would entail per cell, the chances of a given immature variant arising devoid of reactive sites for self components would seem to be extremely small. It follows that elimination of all cells with self-affinities would mean destruction of the majority, rather than the minority, of the immature variants. In this instance it is surely easier to envisage elimination of the self-reactive groups themselves, rather than of the variants possessing such groups.

The hypothesis advanced here does not have any special advantage over most of the recent theories of antibody production; like them, it is neither proved by, nor does it conflict with, the known facts. It is hoped, however, that it succeeds in emphasizing the need for more comparative studies, especially on very primitive metazoa. Metchnikoff wrote ". . . l'étude de ces animaux (inférieurs), que nous présentent des conditions bien plus simples et plus primitives que l'homme et les vertebrés, nous fournit pour ainsi dire la clef des phénomènes pathologiques compliqués"[27]. Although antibody production is not itself a pathological phenomenon, these words are nevertheless very much to the point; in relation to the problem of specific acquired immunity they are as meaningful to-day as in 1892 when they were written.

1 Ehrlich, P., *Deutsch. med. Wschr.*, **24**, 597 (1898).
2 Ehrlich, P., *Proc. Roy. Soc.*, B, **66**, 424 (1900).
3 Jerne, N. K., *Proc. U.S. Nat. Acad. Sci.*, **41**, 849 (1955).
4 Talmage, D. W., *Science*, **129**, 1643 (1959).
5 Breinl, F., and Haurowitz, F., *Z. physiol. Chem.*, **192**, 45 (1930).
6 Pauling, L., *J. Amer. Chem. Soc.*, **62**, 2643 (1940).
7 Burnet, F. M., and Fenner, F., "The Production of Antibodies" (Macmillan and Co., Ltd., Melbourne, Australia, 1949).
8 Grabar, P., Abstracts of Communications. First Intern. Cong. Biochem., 446 (Cambridge, 1949).
9 Monod, J., in "Cellular and Humoral Aspects of the Hypersensitive States", ed. H. S. Lawrence, 323 (Hoeber-Harper, New York, 1959).
10 Tyler, A., *Physiol. Revs.*, **28**, 180 (1948).
11 Spiegel, M., *Ann. N.Y. Acad. Sci.*, **60**, 1056 (1955).
12 Tyler, A., *Growth*, **10** (Supp.), 7 (1947).
13 Weiss, P., *Yale J. Biol. Med.*, **19**, 235 (1947).
14 Weiss, P., *Quart. Rev. Biol.*, **25**, 177 (1950).
15 Ray, D. L., *J. Exp. Zool.*, **118**, 443 (1951).
16 Humphrey, J. H., and Mota, I., *Immunol.*, **2**, 19 (1959).
17 Boyden, S. V., Sorkin, E., and Spárck, J. V., Symposium on the Mechanism of Antibody Formation, Liblice, Czechoslovakia, 1959 (in the press).
18 Boyden, S. V., and Sorkin, E. (submitted for publication).
19 Pollock, M. R., in "Adaptation in Micro-Organisms", Third Symposium of the Society for General Microbiology (Cambridge University Press, 1953).
20 Porter, R. R., *Nature*, **182**, 670 (1958).
21 Lederberg, J., *J. Cell. Comp. Physiol.*, **52**, Supp. 1, 398 (1958).
22 Lederberg, J., *Science*, **129**, 1649 (1959).
23 Burnet, F. M., *Austral. J. Sci.*, **20**, 66 (1957).
24 Burnet, F. M., "The Clonal Selection Theory of Acquired Immunity" (Cambridge University Press, 1959).
25 Talmage, D. W., "Ann. Rev. Med.", **8**, 239 (1957).
26 Pappenheimer, jun., A. M., Scharff, M., and Uhr, J. W., in "Mechanisms of Hypersensitivity", eds. J. H. Shaffer, G. A. LoGrippo and M. W. Chase, 417 (Little, Brown and Co., Boston, 1959).
27 Metchnikoff, E., "Pathologie Comparée de L'Inflammation" (G. Masson, Paris, 1892).

VARIATION OF UPPER-ATMOSPHERE DENSITY WITH LATITUDE AND SEASON: FURTHER EVIDENCE FROM SATELLITE ORBITS

By D. G. KING-HELE and Mrs. D. M. C. WALKER

Royal Aircraft Establishment, Farnborough, Hants

IN a recent article in *Nature*[1], it was explained how the variations in air density at heights and latitudes near that of the perigee of an Earth satellite could be found from the rate of contraction of the orbit of the satellite. The method was applied to *Sputnik* 3 (1958β2), and it was concluded that, for latitudes between 50° N. and 65° S., the air density at a height of 226 km. did not depart from its average value by a factor of more than 1·5 as a result of variations with latitude and season, and all other causes, such as solar disturbances[2–6] (which give rise to an oscillation with an amplitude of about ± 20 per cent) and a possible, much smaller, day-to-night effect. This conclusion conflicts with earlier direct measurements from rockets[7,8], which suggested a much wider variation with latitude and season, by a factor of 5 or even 10, at heights of 200–300 km. Since the subject remains rather controversial, it seems worth giving further results which have recently become available.

The latitude-range covered by *Sputnik* 3 (50° N.–65° S.) can be extended with the aid of *Discoverer* 6 (1959ζ), the orbit of which was inclined at 84° to the equator. *Discoverer* 6 was in orbit for two months, from August 19 to October 20, 1959, and during this time its perigee moved northward from a latitude of about 50° N. to 84° N., and then southward to about 50° S. The air density at the initial perigee height of 220 km., obtained by the method previously described[1], and expressed as a multiple σ of the average density, is plotted in Fig. 1. The points in Fig. 1 are at intervals of 6 days, during which time

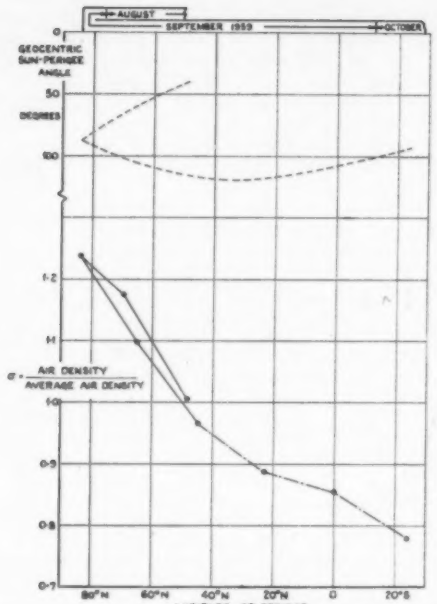


Fig. 1. Air density at a height of 220 km., deduced from the orbit of *Discoverer* 6: variation with latitude and time

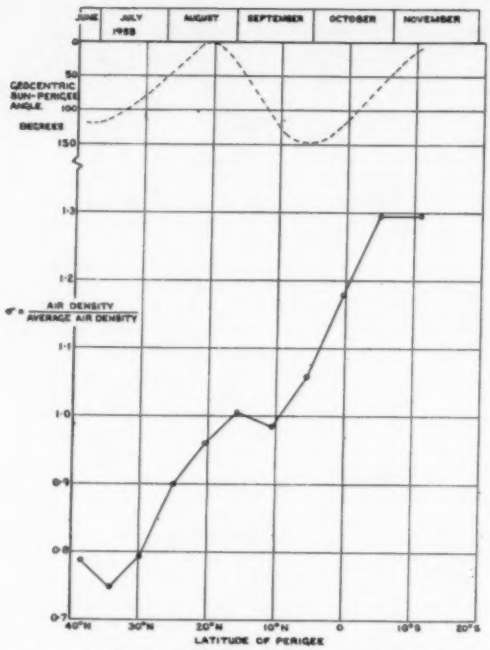


Fig. 2. Air density at a height of 220 km., deduced from the orbit of *Sputnik* 3 rocket: variation with latitude and time

the perigee latitude changes by about 20°. The main orbital data—the values of orbital period—were obtained partly from information issued by Project Space Track, Bedford, Mass., and partly from observations by the authors. Errors in the values plotted in Fig. 1 may arise from (1) errors in the orbital data, (2) errors in the assumed value for atmospheric scale height[1], and (3) changes in the mode of rotation of the satellite. The estimated standard deviation in Fig. 1 from these causes is a little more than 10 per cent.

Similar calculations have been made for the rocket of *Sputnik* 3 (1958δ1), and the results are plotted in Fig. 2. This satellite[4] revealed strong and regular variations in air density, with amplitude about ±20 per cent and having maxima at intervals of about 28 days and minima at similar intervals. The points in Fig. 2 were calculated at intervals of 14 days, halfway between a maximum and a minimum, to smooth out these oscillations. The standard deviation in Fig. 2 is likely to be about 10 per cent, and again the relevant perigee height is 220 km.

The graphs in Figs. 1 and 2 represent, in effect, an average density over the range of latitudes near perigee where the air drag is important. For *Sputnik* 3 rocket the initial orbital eccentricity was near 0·1, and 80 per cent of the total drag effect occurred at latitudes within 15° of perigee. But *Discoverer* 6 had a lower initial eccentricity, 0·047, and the drag effect is therefore spread over a wider range of latitude, 80 per cent of it occurring at latitudes within 30 degrees of perigee. Towards the end of the life

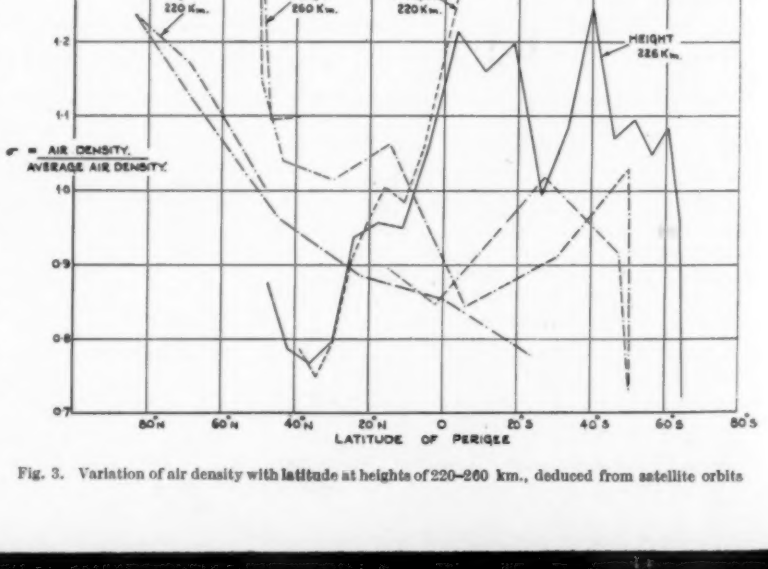

1958 δ2 SPUTNIK 3
1958 δ1 SPUTNIK 3 ROCKET
1958 ε EXPLORER 4
1959 ζ DISCOVERER 6
HEIGHT 220 Km.
HEIGHT 260 Km.
HEIGHT 220 Km.
HEIGHT 226 Km.
ρ / ρ̄ = AIR DENSITY / AVERAGE AIR DENSITY
LATITUDE OF PERIGEE

Fig. 3. Variation of air density with latitude at heights of 220–260 km., deduced from satellite orbits

of this satellite the spread in latitude is even greater, and the graph in Fig. 1 has been drawn as a broken line, since it does not represent realistically the detailed variation of density with latitude, though it does serve to indicate the average density in the equatorial region, for comparison with the density in north polar regions on the left of the graph.

Figs. 1 and 2 both confirm the earlier evidence that density does not depart from its average value, at heights near 220 km., by a factor of more than 1·5, and suggest that the variation due to latitude and season is probably a good deal smaller than this.

The angle *SCP* subtended at the centre of the Earth *C* by the sun *S* and perigee *P* of the satellite is also plotted in Figs. 1 and 2. If *SCP* exceeds 90°, the Earth below perigee is in darkness; if *SCP* exceeds about 108°, the perigee point itself is in darkness. The slight suggestion of a small variation in density between day and night indicated[1] by *Sputnik* 3 is given little support by Figs. 1 and 2. At heights around 220 km. it seems probable that day-to-night changes in density do not exceed ± 10 per cent.

The values of density at heights of about 220 km. given by *Sputnik* 3 (ref. 1), *Discoverer* 6 (Fig. 1) and *Sputnik* 3 rocket (Fig. 2) are plotted against latitude in Fig. 3, together with similar results for heights near 260 km. obtained from *Explorer* 4 (1958ε) by Schilling and Whitney[9] (though the method of presentation of these latter results has been altered, to fit in with the scheme of Fig. 3). The first point of interest in Fig. 3 is the close agreement between the results from *Sputnik* 3 and its rocket. This is to be expected because the perigees of the two satellites reached a given latitude at roughly the same dates and were therefore sampling the same regions of air. The small difference, averaging about 4 per cent, between the two sets of values suggests that the error inherent in the method of calculation is less than the 10 per cent mentioned earlier.

Apart from this close correspondence between the results from *Sputnik* 3 and its rocket, Fig. 3 is notable for an almost complete absence of significant structure: the only positive conclusion which might possibly be drawn is that density tends to be rather high north of lat. 50° N. and rather low south of lat. 60° S. On the whole, however, it appears more likely that the variations are unconnected with latitude. When the values of σ are plotted against the difference between perigee latitude and the declination of the Sun, to indicate possible seasonal effects, the resultant graph, which is not reproduced here, resembles Fig. 3 and again shows no sign of significant structure. The obvious conclusion is that the variations in density are controlled by time-dependent sources, for example, solar disturbances, rather than by latitude and season, which depend essentially on the position of the satellite relative to the centre of the Earth.

To sum up the results of this and previous work, it appears that solar disturbances are the main factor influencing the air density at heights of 200–300 km., giving rise to variations which have an amplitude of about ± 20 per cent and a periodicity of about 28 days. Day-to-night changes may also possibly have an effect, with the density not more than 10 per cent above average by day. Variations with latitude and season are small, and, when density is plotted against latitude or season, no consistent pattern emerges. At greater heights, 300–700 km., the variation from day to night is much more important[10,11] and has a greater amplitude than the 28-day variation due to solar disturbances.

[1] King-Hele, D. G., *Nature*, **184**, 1267 (1959).
[2] Nonweiler, T. R., *Nature*, **182**, 468 (1958).
[3] Jacchia, L. G., *Nature*, **183**, 526 (1959).
[4] King-Hele, D. G., and Walker, D. M. C., *Nature*, **183**, 527 (1959).
[5] Priester, W., *Naturwiss.*, **46**, 197 (1959).
[6] Jacchia, L. G., *Nature*, **183**, 1662 (1959).
[7] Kellogg, W. W., *Planet. Space Sci.*, **1**, 71 (1959).
[8] LaGow, H. E., Horowitz, R., and Ainsworth, J., paper presented at Fifth CSAGI Assembly (Moscow, 1958).
[9] Schilling, G. F., and Whitney, C. A., *Planet. Space Sci.*, **1**, 136 (1959).
[10] Jacchia, L. G., Smithsonian Astrophys. Obs. Spec. Rep., 29 (1959).
[11] Martin, H. A., and Priester, W., *Nature*, **185**, 600 (1960).

THE APPROACHING DESCENT OF *SPUTNIK* 3 (1958δ2)

By B. R. MAY

Radio Research Station, Department of Scientific and Industrial Research, Slough, Bucks

SINCE *Sputnik* 3 (1958δ2) is nearing the end of its life-time, it may be of some interest to give a short account of its progress since the last review[1], and to discuss the likelihood of favourable observing periods from the British Isles, before its descent.

Throughout May, most of July, and August, the satellite was illuminated by the Sun on at least one transit each night, and on some nights as many as four consecutive transits were visible from the British Isles. This was due to the high declination of the Sun and the comparatively great heights (925 km., and above) at which the satellite traversed our latitudes. The fine weather enabled visual observers to obtain regular sightings, at stellar magnitudes ranging from + 3 to + 9. During June, however, the near transits occurred in the day-time, so that only radio observations were possible during that month. Since about the end of August the transits have been subject to varying eclipse conditions, and the number of visual observations made has generally decreased due to this, and to a greater extent, to the unfavourable weather conditions. Because of the rotation of the perigee of this orbit towards the northern hemisphere, the height of transit over our latitudes has been steadily decreasing since the end of June 1959, when apogee was at the maximum northern latitude. At the time of writing (February 15), the height of the satellite over our latitudes ranges from 220 to 470 km.

Since the perigee distance of this satellite is fairly small—about 6,560 km.—the magnitude of the atmospheric drag experienced by the satellite at its perigee will change appreciably as the perigee rotates over the oblate Earth. Thus, the estimation of the life-time of the satellite, which is based largely upon the drag, is subject to uncertainties even after the orbit is well known. In May 1959 a theoretical date of descent in early January 1960 was indicated—the present estimate places it in late March 1960.

For most of the few remaining weeks of the life of the satellite the near transits will take place in the day-time and, since the transmitters of the satellite are powered by solar cells only, good opportunities for radio tracking will be afforded. Transits for visual observations from the British Isles will occur from about March 16 onwards, in the mornings. Due to its low height, *Sputnik* 3 may well become a comparatively bright object, and it is hoped that this will increase the chances of valuable observations being made upon the last revolutions of this most interesting and successful satellite.

This communication is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research.

[1] May, B. R., and Smith, D. E., *Nature*, **184**, 765 (1959).

THE CANADIAN COMMITTEE ON OCEANOGRAPHY

THE Canadian Committee on Oceanography represents the re-organized Canadian Joint Committee on Oceanography which came into existence on April 1, 1946. It is comprised of representatives of the following Government agencies: Royal Canadian Navy, Fisheries Research Board, Department of Mines and Technical Surveys, Defence Research Board, National Research Council, Meteorological Branch of the Department of Transport, and Marine Services of the Department of Transport. In addition there are representatives of academic interests in oceanography nominated by the National Research Council.

The Committee is under the chairmanship of Dr. W. E. van Steenburgh, director general of Scientific Services, Department of Mines and Technical Surveys, Ottawa, Ontario. The secretary is Dr. H. B. Hachey, chief oceanographer, Fisheries Research Board of Canada, Ottawa, Ontario.

The functions of the Committee are to: (*a*) co-ordinate the oceanographic activities of all Canadian Government agencies; (*b*) act, as designated by the National Research Council, as the national committee for the Scientific Committee on Oceanographic Research; (*c*) act, as designated by the National Research Council, as the Section on Oceanography of the Council's Associate Committee on Geodesy and Geophysics.

Each of the co-operating organizations is either actively engaged in oceanographic investigations or contributes in ships, laboratory facilities, personnel or funds towards the Canadian programme, the total effort being co-ordinated through the Committee.

Activities coming under the Canadian Committee can be divided according to regions under investigation.

(*a*) *Atlantic coast.* This includes general surveys of the Gulf of St. Lawrence, the Scotian Shelf, the Bay of Fundy region, Newfoundland waters, and the Labrador and sub-Arctic seas; studies on slope waters and the mechanism of the Gulf Stream system, on the fundamentals of the Gaspe Current and Cabot Stream, on tides and currents, on cyclic variations in water temperatures, on problems of the varying influence of Atlantic and Arctic waters, on the formation and movements of ice, on biological, geological and geophysical problems, and special studies involving fisheries, naval and other national problems.

(*b*) *Pacific coast.* The work here covers general surveys of the waters of the eastern North Pacific, the Straits of Georgia and Juan de Fuca, Hecate Strait, and the fjord-like British Columbia inlets; studies on pollution in the sea as related to pulp-mill effluents and sewage; studies of current patterns and tides in Nature, and investigations of the fundamentals of flow by means of models; productivity studies, and variations in water characteristics as followed by observations at a network of coastal stations and from the Canadian Weathership; studies on biological, geological and geophysical problems and special studies involving fisheries, naval, and other national problems.

(*c*) *Arctic coast.* General surveys are being made of the waters of the Canadian Arctic, extending from the Bering Strait and Beaufort Sea to Baffin Bay and Davis Strait, with particular attention to the water characteristics in the various sub-areas and the volume and direction of flow through the various passages. An extensive survey is being undertaken of the Polar Continental Shelf, involving general oceanographic observations, tidal and current studies, geological, geophysical and biological investigations.

The work of the Committee is being carried out at a number of institutions. There is an Institute of Oceanography at the University of British Columbia, Vancouver, B.C., under the directorship of Dr. George Pickard, and an Institute of Oceanography at Dalhousie University, Halifax, N.S., has recently been set up under the directorship of Dr. F. R. Hayes.

The Pacific Naval Laboratory at Esquimalt, B.C., and the Naval Research Establishment at Dartmouth, N.S., operated by the Defence Research Board, conduct oceanographic research and surveys in support of military requirements.

Plans for the Bedford Institute of Oceanography, near Halifax, N.S., have been announced and the development is now under way. The Institute, which is to be completed in five years, will have a staff of 300 oceanographers, hydrographers, submarine geologists and other scientific personnel, plus supporting staff, and an operating fleet of ten oceanographic vessels. The Atlantic Oceanographic Group of the Fisheries Research Board will also be located in the Institute. This Institute will be under the Department of Mines and Technical Surveys. In support of its work, a substantial shipbuilding programme is planned. The first ship, the C.G.S. *Hudson*, which is expected to cost 7 million dollars, is to be commissioned in 1961. Dr. W. M. Cameron has recently been appointed to take charge of the development of the Bedford Institute. Widely known for his work in oceanographic research, Dr. Cameron has made special studies in the fields of biological and anti-submarine research. He is best known, however, for his Arctic studies and for his work in theoretical oceanography, particularly the theory of circulation in estuaries. His Arctic studies were mainly carried out as a member of the joint Canada–United States Beaufort Sea Expeditions, in which he was senior Canadian scientist. He was for a time professor of oceanography in the University of British Columbia, where he established an Institute of Oceanography. Since 1958 he has been director of plans of the Defence Research Board in Canada.

BIRDS AND FORESTS

THE Council of the British Trust for Ornithology has recently taken the initiative in promoting discussions with other bodies interested in economic aspects of ornithology. The second of its series of joint conferences was held at Northerwood House, Lyndhurst, Hampshire, during October 30–November 1. The Forestry Commission was the Trust's partner in organizing the conference, and the subject was "Birds and Forests".

In opening the conference, Mr. E. M. Nicholson (Nature Conservancy) reviewed the relations between birds and forestry. He pointed out that remarkably little experimental work had so far been done in Britain to assess the part played by birds in the forest economy; the work of the Edward Grey Institute of Field Ornithology on tits and other birds in the Breckland and the Forest of Dean, and of H. N. Southern, of the Bureau of Animal Population, on the tawny owl in Wytham Wood, were examples of what could be done. He called for a new look at the problems of forest ornithology as a whole.

Mr. T. R. Peace (Forestry Commission) referred to the recent shift of emphasis from the idea of creating a strategic reserve of timber to that of economic forestry. This meant turning away from the natural forest towards a greater degree of deliberate tree farming. The Commission, as its creation of National Forest Parks has shown, was always prepared to pay attention to amenity; but economic considerations do not permit forests to be designed primarily for the encouragement of wild life. The Forestry Commission had not so far undertaken any research on problems of forest ornithology because it did not believe a case had yet been made out for birds being sufficiently beneficial to woodlands under British conditions to justify this.

In the subsequent discussion the point was brought out that the forester was faced with a choice between the small but continuing expense of providing and maintaining nest-boxes in order to increase the population of pest-predator birds and the much greater, but only very occasional, expense of attacking an infestation with sprays.

Mr. A. R. Mitchell (Forestry Commission) described the impact of forestry practice on bird life in woodlands. Wood-larks, for example, found forest nurseries a suitable habitat. On the other hand, the first thinning of a new plantation removed the forked and crooked trees in which birds liked to nest.

Dr. J. A. Gibb (Animal Ecology Section, Department of Scientific and Industrial Research, New Zealand; formerly of the Edward Grey Institute of Field Ornithology, Oxford) sent a paper describing the work of the Breckland Research Unit of the Edward Grey Institute with special reference to populations of insectivorous birds and their food supply in pine plantations. The Unit was able to demonstrate that the density of tits and goldcrests in the Breckland pine plantations (mainly 20–30 years old) in winter varied closely with their stock of food. In severe winters, the tits and goldcrests might be actually consuming 40 per cent of the total amount of insect food available for them, and very much larger proportions of the available supplies of their favourite foods. Though this did not by itself prove that the birds' numbers were being controlled in a density-dependent way by food shortage, it did prove the existence of the indispensable condition needed for this method of control to operate.

The result of the Breckland work was to suggest that the provision of nest-boxes, though increasing the local density of birds in the breeding season, could not enable a greater number of tits to survive the crucial winter season in any particular forest. It might indeed be more to the point to increase the stock of winter food available for the birds by planting suitable trees and shrubs on the forest margins to diversify the supply; for example, spruce and beech would provide autumn stocks of food for coal tits, which they could store for use in the winter (pines shed their seeds in spring).

As regards the impact of birds on insect populations, the Breckland researches showed that bird predation accounted for a substantial proportion of insect stocks, so that birds are a force to be reckoned with in forest economics.

Dr. Herbert Bruns (Staatliche Vogelschutzwarte, Hamburg) described various aspects of forest research on economic ornithology in Germany. Food studies showed that, on the whole, insect-eating birds fed chiefly on pest-insects, sometimes in large quantities. This did not, however, necessarily prove that they were important controls on these insects. However, there were several convincing examples in Germany where it could be proved that birds had actively controlled insect pests. In one pine forest in Baden it had been shown that insect infestation was much worse in nearly birdless monocultural plantations than in bird-rich woods nearby. When more nest-boxes were brought into the wood, the infestation was reduced.

Summing up, Dr. Bruns said that birds were not a panacea for all forest ills; but an important link in the natural chain of factors making for a healthy forest. Methods of sylviculture were most important, and it must be borne in mind that most German forests were much more mature than the average British forest. Much of the different British experience might be due to the immaturity of many British forests. The critical age for pest-devastation came at 60–70 years.

Mr. R. K. Murton (Ministry of Agriculture) discussed the control of wood-pigeons, which might be a serious forest pest, especially to the leading shoots of Sitka spruce and other species. In East Anglia at least the Forestry Commission could be absolved from generating a high population of wood-pigeons, for their plantations were too extensive. Dense nesting of wood-pigeons only occurs in a fringe about 100 yards deep around the edges of large blocks of woodland, since the birds are unwilling to nest very far from their feeding-grounds. Wood-pigeons therefore prefer to breed in hedgerows, shelter-belts and the small spinneys planted as shelter for game. The game preserver indeed was largely responsible for the existence of the plague of wood-pigeons in East Anglia, for in addition to providing their nesting places, he prevented any control measures until February, after the shooting season, when much damage had already been done to the crops of farmers and foresters. Moreover, it was the keeper who killed off the two principal predators of the wood-pigeon,

the jay and the magpie, which are responsible for 80 per cent of the losses of wood-pigeons' eggs.

Dr. Bruce Campbell (British Trust for Ornithology) discussed population problems in a typical insect-eating forest bird, the pied flycatcher, illustrated by the ringing and nest-box experiments he was undertaking in the Forest of Dean.

Mr. W. A. Cadman (Forestry Commission) described the progression of wild life at various stages in the development of forests. He said that the pine marten had increased in some Welsh forests.

Mr. Nigel Gray (Imperial Chemical Industries Game Research Station) discussed the relations between game preservation and forestry. He said that good forestry made for good game habitat; but it was essential to have a reasonable mixture of tree species. If the Forestry Commission were to appoint game advisers, it would succeed in greatly boosting private forestry, by showing owners how it could be combined with game preservation.

Dr. I. D. Pennie (British Trust for Ornithology) spoke about the capercaillie and its effects on woodlands. Foresters claimed that in autumn and winter the capercaillie ate almost exclusively the shoots and buds of conifers; but in fact it seemed much to prefer the shoots of transplanted trees in nurseries and plantations to those of naturally regenerating trees. It was thus the shift in forestry practice, from the natural woodland to the artificial plantation, that had transformed this normal member of the woodland avifauna of northern Europe into a forest pest in parts of Scotland.

There were two field excursions during the conference, one under Mr. B. Gale, with a forestry angle, and one under Mr. Edwin Cohen, mainly for bird-watchers.

R. S. R. Fitter

OBITUARIES

Mr. E. Price Evans

Mr. Evan Price Evans died on April 27, 1959, at his home in Eastbourne, aged seventy-seven. He will be remembered for the part he played in introducing the teaching of plant ecology into schools; it is probably quite largely due to him that at least in some schools ecological teaching on scientific lines has taken the place of mere 'Nature study'.

Price Evans was a Welsh-speaking Welshman, born at Corris in Merioneth on January 19, 1882. Educated in his native village and at Towyn County School, he entered the University College of North Wales, Bangor, in 1900 and was trained to be a teacher. There he attended the botanical lectures of Prof. Reginald Phillips and Dr. (later Prof.) J. Lloyd Williams. His first appointment was at his own school at Towyn, under the headmastership of a notable chemist, Thomas Jones.

At Towyn he had already experimented with teaching field botany even to quite junior forms, and he later developed this teaching when holding a post at Ryhope, Co. Durham. In his youth, however, he was better known as a footballer than as a scientist and at one time played for Wales in the amateur internationals. From Ryhope he went to Warrington, where he became headmaster of the Grammar School.

At Ryhope from 1913 onwards he began teaching ecology and vegetation mapping to his classes, and his efforts in this direction led him to correspond with the late Sir Arthur Tansley, who was then at Cambridge. Tansley encouraged him to publish an account of his teaching methods in the *School Science Review*, and later they wrote in collaboration "Plant Ecology and the School" (1946).

Price Evans was a keen student of vegetation on his own account, especially that of his native mountains, which he knew very intimately. He contributed several papers to the *Journal of Ecology*, of which one dealing with Cader Idris is still the only published account of the vegetation of a geologically and botanically very interesting area and is often quoted. He was an accurate observer and kept notes methodically; his unpublished note-books are full of valuable observations on the plants and plant communities of North Wales. The University of Wales recognized his work by awarding him the honorary degree of M.Sc. in 1933.

During and after the Second World War, Price Evans was a keen supporter of Nature conservation, and he was an active member of the Nature Conservancy's Committee for Wales from its inception until shortly before his death. He had a vigorous and friendly personality and will be much missed.

P. W. Richards

Dr. Z. K. A. Moszynski

Dr. Z. K. A. Moszynski, a principal scientific officer of the British Coke Research Association, died at his home in Chesterfield, Derbyshire, on February 13. Zbigniew Konrad Antoni Moszynski was born at Lwow, Poland, on December 16, 1907, and studied in the Department of Chemical Engineering, University of Lwow (1927–34), where he took his diploma (Dipl. Ing.). During 1934–35 he worked in the Laboratory of Potassium Salts, University of Lwow. During 1935–39 he was deputy inspector for civil defence of industry, Polish Ministry of Defence, in the County of Lwow.

In August 1939 he was mobilized and served in the Polish–German campaign, escaping to Hungary, where he was interned. For five months he was assistant to the professor of agricultural chemistry, University of Magyarovar, but in March 1940 he joined the Polish forces in France, being evacuated to England. On behalf of the Polish General Staff he carried out research work in the Mining Department, University of Birmingham, between 1944 and 1945 on the use of carbonized peat/coal blends for mobile gas generators. During 1946–47 he continued and extended this work on the production of reactive carbonized fuels in the Fuel Department, University of Leeds, and obtained the degree of doctor of philosophy.

In September 1947 he was demobilized and in the same year became a British subject. He joined the staff of the British Coke Research Association, at the Midland Coke Research Station, where he was in charge of personnel and acted as secretary of a

research group working on the analysis of coal and coke. His bomb combustion method for the determination of both sulphur and chlorine in coal is well known to fuel scientists, and is to become an international standard method. With the building of the new Coke Research Centre at Chesterfield in 1958 he transferred his home to Derbyshire and took up new work for the British Coke Research Association dealing with the problems of air and water pollution.

NEWS and VIEWS

Physiology at Oxford : Prof. E. G. T. Liddell, F.R.S.

THE near prospect of Prof. E. G. T. Liddell's retirement from Oxford's Waynflete professorship of physiology has aroused a widespread interest among physiologists everywhere and, indeed, among all those who are concerned for the advancement by experimental research of knowledge in the medical range of the sciences. For there can be no doubt that the Waynflete chair, during the tenures of a succession of distinguished occupants, has achieved and maintained a reputation second to that of no other chair of physiology, anywhere in the world. Sir John Burdon-Sanderson and his pupil and successor, Prof. Francis Gotch, made contributions of fundamental importance to knowledge of the nature of nervous impulses, as excited and propagated in the continuity of nerve fibres. Under Sir Charles Sherrington the reputation of the Waynflete chair, and of the Oxford School of Physiology attached to it, attained additional eminence, through the discoveries which he and a series of distinguished associates made concerning the intricate phenomena of the central nervous system, involving the transmission of nervous excitations across systems of synaptic junctions of varying complexity; discoveries which have provided a large component of the foundation for modern neurological theory and practice. It is in this field of research, on the physiological processes of the brain and the spinal cord, that Prof. Liddell, as an intimate pupil of and collaborator with his great predecessor, has maintained that distinguished tradition.

Sir Lindor Brown, C.B.E., F.R.S.

UNDER Sir George Lindor Brown, Jodrell professor of physiology in University College, London, whose appointment to succeed Prof. Liddell in the Waynflete chair has recently been announced, it may be confidently expected that the tradition will be further maintained. After a distinguished student career in physiology at Manchester, Brown held a lectureship at Leeds, where the late B. A. McSwiney was then the professor. Work there, with McSwiney and others, gave early evidence of Brown's interest in the special physiology of the autonomic nervous system, and in the then recent evidence for a chemical mechanism of excitatory transmission at its ganglionic and peripheral junctions. With a grant from the Medical Research Council, he also worked for a period in Sherrington's Oxford department with J. C. Eccles. He then, in 1934, accepted an invitation to join Sir Henry Dale's team at the National Institute for Medical Research, and played a prominent part in extending the evidence for a cholinergic mechanism, to the transmission from motor nerve endings to the end-plates of voluntary muscle. Brown continued investigations in this general field with a series of distinguished colleagues and visiting experts after Sir Henry's retirement in 1942, having meanwhile become engaged in important war-time researches and responsibilities. In 1949 he became professor of physiology in University College, London, where another famous chair of physiology will be vacated by his removal to Oxford; and since 1955 he has been the biological secretary of the Royal Society. The chief focus of research-interest in the Oxford School of Physiology may, perhaps, move for a time from the central towards the peripheral nervous system; but there need be no fear of a lowering of its standard.

Astronomy at Oxford : Prof. H. H. Plaskett, F.R.S.

ON September 30, Prof. H. H. Plaskett retires under the age limit, after twenty-eight years as Savilian professor of astronomy and director of the University Observatory at Oxford. His tenure of office has been notable for the building up of a school of solar physics, now well known for the thoroughness of its methods, the clarity of exposition in its lectures, and its avoidance of popular publicity. With only modest resources, two new solar telescopes and large spectrographs have been built and operated successfully. Prof. Plaskett's own work has been both observational and theoretical. He has studied models of the solar atmosphere, the formation of Fraunhofer lines, the structure of the solar granulation and problems of the chromosphere. Under his inspiration and guidance his colleagues and pupils have also made important contributions to solar physics. Among them they have discovered the strengthening of weak Fraunhofer lines from centre to limb, they have invented elegant interferometric methods for measurement of Fraunhofer and chromospheric lines, developed improvements in the mathematics of stellar atmosphere theory, and made long series of precision measurements to try to find whether the Einstein red-shift can be detected in the solar spectrum. Finding that the red-shift is obscured by spectrum line displacements arising from other causes, they have been led on one hand to study velocity fields in the solar photosphere, and on the other to make laboratory measurements of pressure shifts in atomic spectra of astronomical importance. Astronomy could well do with more of the devotion to careful measurement and the restraint on speculation which have characterized Oxford work under Prof. Plaskett.

Prof. D. E. Blackwell

DR. D. E. BLACKWELL, who is to succeed Prof. Plaskett, has been assistant director of the Solar Physics Observatory, Cambridge, since 1948. He entered Sidney Sussex College in 1943 as a major scholar, from the Merchant Taylors' School, took first-class honours in both parts of the Natural Sciences Tripos, was an Isaac Newton Student in 1946 and Stokes Student at Pembroke College in 1947. His astronomical work has dealt chiefly with

the solar corona and the zodiacal light. In a series of well-planned, adventurous and successful expeditions, in the Sudan for the 1952 eclipse, in an open aircraft at 30,000 ft. for the 1954 eclipse, over the Pacific in another aircraft in 1955, in the high Andes in 1958, he has extended optical measures until we now have reliable curves of brightness and polarization from the inner corona to the zodiacal light region 70° away. With M. F. Ingham he has photographed the zodiacal light spectrum with higher resolution than hitherto attained; their results have considerably altered our views on electron densities in interplanetary space and on the light scattered by interplanetary dust. They have also shown beyond doubt that the zodiacal light is sometimes variable. In 1956, and again in 1957, Blackwell and A. Dollfus made pioneer photographs of solar granulation from a balloon at 15,000–20,000 ft., using an 11-in. telescope, and demonstrated that most of the loss of telescope definition from bad day-time seeing can be avoided in this way. Dr. Blackwell is one who believes that observational astronomers must at all times seize upon any new techniques which promise significant advantage over older methods, and has acted on this belief with notable success.

Biology at Melbourne (Monash): Prof. A. J. Marshall

Dr. A. J. Marshall, reader in zoology and comparative anatomy in the University of London at St. Bartholomew's Hospital Medical College, where he is head of the Department, has been appointed to the chair of biology in the new Monash University in Melbourne, Australia. Dr. Marshall is an Australian by birth and upbringing; soon after entering the University of Sydney he joined the Oxford University Expedition to the New Hebrides, and then went to Oxford to help work out the results before returning to Sydney to finish his course and to graduate. During 1941–45 he was engaged in military service in northern Australia and New Guinea, and commanded the independent infantry "Jockforce" in the Wewak Campaign. In 1946 he returned to Oxford, where he obtained his D.Phil. and, later, D.Sc. degrees. In 1949 he resigned his Beit Memorial Research Fellowship to accept his appointment at St. Bartholomew's.

Dr. Marshall has always been a naturalist, and in his early years published a number of papers on natural history subjects in Australian journals. Under academic influence, and the guidance of Dr. John R. Baker and the late Dr. F. H. A. Marshall, his research interests concentrated on the physiology of reproduction in vertebrates, especially in its relation to behaviour, migration, and the ecology of breeding seasons. He has been strikingly successful in combining field and laboratory work by using the changing environment as a kind of natural laboratory. Key localities and situations, such as deserts, the arctic, early springs, and unusually severe winters, have been selected, and free-living birds, fishes, reptiles and mammals have been collected at periods, often in successive years, that were expected to yield the most valuable results upon subsequent histological investigation of their sexual cycles. He has always kept one foot in the field while the other was in the laboratory. One of his most important discoveries is the post-breeding metamorphosis of the seminiferous tubules in non-mammalian vertebrates into a mass of cholesterol-positive lipoid material which contains progesterone, and the somatic and psychological effects of the action of this hitherto undescribed endocrine gland. A very long series of papers by Marshall and his colleagues record these and cognate researches, crowned by his penetrating study of reproductive physiology and behaviour of the bower birds published by the Clarendon Press in 1954. Prof. Marshall will not only build up a first-rate teaching department at Monash, but will also undoubtedly establish a school of research in field-endocrinology, for which Australia offers unbounded opportunities, that will redound both to his own credit and that of his colleagues in the Old Country who first guided his steps towards this rewarding subject of research.

Cyto-Genetics at the Royal Botanic Gardens, Kew: Dr. Keith Jones

Dr. Keith Jones, a graduate of the University College of Wales, Aberystwyth, has been appointed a principal scientific officer at the Royal Botanic Gardens, Kew, to undertake research in cyto-genetics. Since 1948 he has been in charge of the Department of Cytology at the Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, where his investigations have been connected mainly with European species of the Gramineae, in particular those of the genera *Agrostis*, *Anthoxanthum*, *Dactylis* and *Holcus*. He was awarded a doctorate by his University in 1957 for his thesis on "Species Differentiation in *Agrostis*", and recently spent a year studying at the Plant Research Institute, Ottawa, as a postgraduate fellow of the National Research Council of Canada.

Although Kew is internationally renowned for the beauty of its Gardens, it is primarily a research establishment concerned with the classification, determination and description of plants. For more than a hundred years its vast collections of herbarium specimens supplemented by living plants have been the subject of intensive morphological and anatomical studies. On the other hand, these unrivalled living collections, estimated to include some 45,000 species and varieties, have received comparatively little attention from the cyto-geneticists, and probably half of them have never been examined cytologically. Here, with such a wealth of desirable material, constantly being enriched by new discoveries, and representative of all groups of vascular plants and of very diverse phytogeographical regions, there is a unique opportunity for extensive cytological and genetical investigations. While the urgent need at present is for karyological studies, especially the determination of numbers and forms of chromosome sets, other lines of research, such as the genetical study of species and putative hybrids impossible of satisfactory classification by ordinary taxonomic methods, are also very necessary. It is hoped that this closer association of systematic botany, cytology and genetics at Kew will lead to the resolving of many difficult taxonomic problems and ultimately to a much improved and more natural system for the flowering plants.

Royal Society of Edinburgh: New Fellows

At a meeting of the Royal Society of Edinburgh, held on March 7, the following were elected Fellows of the Society: Dr. I. R. C. Batchelor, physician superintendent, Dundee Royal Mental Hospital, and head of the Department of Psychiatry, University of St. Andrews; Dr. D. Bijl, lecturer in natural philosophy, University of St. Andrews; Prof. T. L. Cottrell, Department of Chemistry, University of

Edinburgh; Prof. R. E. Coupland, Department of Anatomy, Queen's College, Dundee; J. A. Crichton, head of the Applied Nutrition Department, Rowett Research Institute, Bucksburn, Aberdeen; Prof. K. W. Donald, Department of Medicine, University of Edinburgh; Dr. W. J. Eggeling, conservation officer for Scotland, Nature Conservancy; Prof. R. B. Fisher, Department of Biochemistry, University of Edinburgh; R. S. Glover, officer-in-charge, Scottish Marine Biological Association's Oceanographic Laboratory, Edinburgh; W. Hutton, deputy-chairman of the South of Scotland Electricity Board; T. R. M. Lawrie, district geologist in charge of Highland work, H.M. Geological Survey, Edinburgh; R. D. Lord, senior lecturer in mathematics, Royal College of Science and Technology, Glasgow; Dr. J. A. Lovern, head of the Lipids and By-products Section, Torry Research Station, Aberdeen; Dr. D. M. C. MacEwan, lecturer in physics, Queen's College, Dundee; Prof. J. L. Malcolm, Department of Physiology, University of Aberdeen; Prof. J. A. Roper, Department of Genetics, University of Sheffield; Dr. F. A. Rushworth, lecturer in natural philosophy, University of St. Andrews; Dr. J. M. Shewan, head of the Bacteriology Section, Torry Research Station, Aberdeen; Prof. M. G. P. Stoker, Department of Virology, University of Glasgow; Prof. P. D. Sturkie, Department of Poultry Physiology, Rutgers University, New Brunswick (presently S.P.S.O., Agricultural Research Council Poultry Research Centre, Edinburgh); Prof. P. A. Sweet, Department of Astronomy, University of Glasgow; Prof. W. J. Tulloch, Department of Bacteriology, Queen's College, Dundee; Dr. G. B. Warburton, senior lecturer in charge of the Postgraduate School of Applied Dynamics, University of Edinburgh; Prof. P. E. Weatherley, Department of Botany, University of Aberdeen; Dr. R. Westwater, manager, Technical Service Department, Imperial Chemical Industries, Ltd. (Nobel Division).

Royal Geographical Society Awards

H.M. THE QUEEN has approved the award of the Royal medals of the Royal Geographical Society as follows: *Patron's Medal*, to Prof. Théodore Monod, director of the Institut français d'Afrique Noire, for geographical exploration and research in the Sahara; *Founder's Medal*, to Phillip G. Law, leader of the Australian National Antarctic Research Expeditions, for Antarctic exploration and research; *Victoria Medals*, to Dr. Armando Cortesão, for studies in the history of Portuguese cartography, and Prof. J. A. Steers, University of Cambridge, for research in coastal morphology; *Murchison Grant*, to Prof. J. Wreford Watson, University of Edinburgh, for contributions to the development of geographical studies in Canada; *Back Grant*, to A. T. Grove, University of Cambridge, for geomorphological studies in Northern Africa; *Cuthbert Peek Grant*, to G. F. Reid, directorate of Overseas Surveys, for contributions to Antarctic surveying; *Gill Memorial*, to Joyce M. Lambert, University of Southampton, for research in the origin of the Broads; and *Mrs. Patrick Ness Award*, to R. J. Adie, University of Birmingham, for contributions to Antarctic geology.

Institute of Metals Awards

THE Institute of Metals has made the following awards: *Institute of Metals (Platinum) Medal* for 1960, to Prof. Robert Franklin Mehl (head of the Department of Metallurgical Engineering, Carnegie Institute of Technology, Pittsburgh) in recognition of his outstanding contributions to the science of metals and to metallurgical education; *Rosenhain Medal* for 1960, to Prof. Norman James Petch (William Cochrane professor of metallurgy, King's College, Newcastle on Tyne) in recognition of his outstanding contributions in the field of physical metallurgy; *W. H. A. Robertson Medal and Premium*, for 1959, to Dr. Maurice Cook and Mr. E. Swainson (formerly chairman and assistant manager, Technical Department, respectively, Imperial Chemical Industries, Ltd., Metals Division, Witton, Birmingham) for a paper on "Arc Melting of Reactive and Refractory Metals", published in the *Journal of the Institute of Metals*, **87**, 161 (1958–59).

Wolfson Research Professorship of the Royal Society

THE Council of the Royal Society has accepted with thanks the offer of the Isaac Wolfson Foundation to create a special fund of £200,000 for the endowment of a research professorship, to be known as the Wolfson Research Professorship of the Royal Society. The chair is to be held by a distinguished British scientist, who will devote the whole of his time to research in such fields as the President and Council of the Royal Society may from time to time think appropriate. The first holder, who has yet to be appointed, will work in the physical sciences or their borderlines with biology. The financial provision allows for adequate scientific and technical assistance for the Wolfson research professor.

Herald Reactor at Aldermaston

THE research reactor *Herald* of the United Kingdom Atomic Energy Authority started operation on March 7 at the Atomic Weapons Research Establishment, Aldermaston. It will be used for nuclear research work requiring intense neutron beams and for irradiation-damage studies on reactor materials. *Herald* is a light water-moderated and -cooled tank-type thermal reactor designed to operate continuously at 5 MW. The core of the reactor is in the form of thin plates of enriched uranium–aluminium alloy suitably spaced in water. A high flux density of thermal and fast neutrons of the order of 10^{14} neutrons per cm.^{2} will be achieved. The reactor was built for the Atomic Energy Authority by the Associated Electrical Industries/John Thompson Nuclear Energy Co., Ltd., and is an adaptation of the *Merlin* reactor.

U.S. Council for the Advancement of Science Writing

A U.S. Council for the Advancement of Science Writing has been established. Its purpose is to stimulate an increase in the quantity and quality of science news that is carried in all media of mass communications—newspapers, television, radio, magazines and books for the general public. It plans to do this by fostering research and training in science journalism, and by sponsoring discussions and seminars on science writing throughout the United States. The aim is to heighten the public's understanding and appreciation of the scientific enterprise. The Council, a non-profit corporation registered in the State of New York, was set up through the efforts of the National Association of Science Writers. Among those elected to the Council at its first meeting in New York City were: *President*, Earl

Ubell, science editor, *New York Herald Tribune*, who also is vice-president of the National Association of Science Writers; *Vice-president*, W. Bradford Wiley, president of John Wiley and Sons; and *Secretary Treasurer*, Prof. Hillier Krieghbaum, Harvard University. Further information can be obtained from the Department of Journalism, New York University.

Scales of Temperature in the Meteorological Office

The Director-General of the Meteorological Office has decided that from January 1, 1961, the celsius (centigrade) scale of temperature will be used for all work carried out within the Office. At present, the Fahrenheit scale is used for surface observations (air temperature, dewpoint, soil and Earth temperatures); the change to the celsius scale for aerological work having been made some time ago. The decision to employ only the celsius scale in professional work is in line with the recommendations of the World Meteorological Organization, and its adoption not only brings the Meteorological Office into line with all other European services but also will greatly facilitate the international exchange of meteorological information. The change will affect only the internal practice of the Meteorological Office, and at present there is no intention to remove the degree Fahrenheit from the published forecasts, or from replies to inquiries.

Sudan Natural History Museum, Khartoum

The report for 1956–58 of the Natural History Museum, Khartoum, is the first to be published since the control of this institution was assumed by the University. Since its formation in the 1920's the Museum has had a chequered career, and it was only recently that it passed to the University of Khartoum. This association has enabled the Museum to draw freely on the specialized knowledge of the University staff and to develop according to a deliberate long-term policy. On the other hand, the Biological Department of the University benefits considerably by easier access to the technical skill and knowledge possessed by the staff of the Museum. It also enables the staff of the University to use freely the collections and records in the Museum for teaching and research. The constitution of the Board for the Natural History Museum still awaits the adoption of a statute by the University Council; but this formality has not prevented full co-operation for some time past.

Communicable Diseases in School

Communicable diseases are one of the commonest causes of school absenteeism. A survey by the World Health Organization examines the statutory regulations in force in fifteen countries (Geneva. 3*s*. 6*d*.). The measures prescribed, of which the exclusion from school of sick children and of contacts is paramount, are compared with the recommendations found in medical literature. Health authorities tend at present to substitute surveillance for stringent measures. Surveillance presupposes well-organized school health services and the existence of bacteriological laboratories. The subject-matter of the study published in the report is arranged under the following headings: legislation, exclusion measures, obligations of parents, teaching staff and health services; measures relating to school staff; and closure of schools. An appendix contains an analysis in tabular form, by country and by disease, of the statutory measures taken in countries the legislation of which is covered by the study. It is surprising to find that there is virtually no uniformity whatever in the measures prescribed for dealing with the occurrence in schools of diseases the epidemiology, evolution and method of spread of which are well known.

Caroline Haslett Exhibition for a Woman Engineer

The Caroline Haslett Memorial Trust announces that the first award to be made by the General Section of the Trust will be an exhibition to enable a woman engineer to visit the U.S.S.R. for the purpose of studying the training, employment and prospects of Soviet women engineers. Applications for the exhibition are invited from women engineers in the United Kingdom whose training and experience would fit them to make the proposed study. The visit will be a short one and will probably take place in the early autumn of this year. After her return to Great Britain the exhibitioner will be required to prepare a report for the Trust and to lecture on her findings. The Caroline Haslett Memorial Trust was established in 1958 to commemorate the work of the late Dame Caroline Haslett. Application forms, which should be returned before March 31, can be obtained from the Secretary, Caroline Haslett Memorial Trust, 25 Foubert's Place, London, W.1.

Horticultural Co-operative Marketing Societies

The Minister of Agriculture, Fisheries and Food, the Rt. Hon. John Hare, is setting up a departmental Working Party "To consider and report on the methods and forms of association between horticultural co-operative marketing societies and their members, and whether these might be modified so as to give the societies more stability and continuity in their supplies of produce". Mr. J. B. Godber, joint parliamentary secretary to the Ministry, will be chairman of the Working Party, and it is expected to have its first meeting in mid-March. It will seek evidence from the agricultural co-operative associations and their constituent societies on the horticultural side, and from other persons with a direct knowledge of the subject.

University News: Bristol

The following are announced: *Appointments*, to become lecturer in aeronautical engineering, Mr. J. W. Flower; to become senior lecturer in physics, Dr. W. M. Gibson. *Grants*, from the Department of Scientific and Industrial Research, £15,000 for an electron microscope, and £3,850 for an investigation in the Department of Physical and Inorganic Chemistry on the electronic spectra of ionized hydrocarbons; from the Royal Society, £2,291 to the Department of Physical and Inorganic Chemistry for the purchase of a high-temperature resistance vacuum furnace in connexion with investigations on the synthesis and chemical properties of carbides, silicides, borides and phosphides of metals. Biological material to the value of £2,800 has been received by the Department of Pharmacology from the Bingham Oceanographic Laboratories, Yale University.

Cambridge

The Council of St. John's College, Cambridge, will make a Kenneth Craik Research Award for the assistance of persons engaged in postgraduate research, preferably in physiological psychology. The value of

the Award will be £450 a year. Applications should be sent to the Master, St. John's College, Cambridge, to reach him not later than April 4.

London

Mr. P. T. Bauer, Smuts reader in Commonwealth studies in the University of Cambridge, has been appointed to the University chair of economics (with special reference to under-developed countries and economic development) at the London School of Economics and Political Science. Mr. C. B. Winsten, lecturer in the Department of Mathematics at the Imperial College of Science and Technology, has been appointed to the University readership in statistics tenable at that College.

Dr. J. H. Green, senior lecturer in physiology in Middlesex Hospital Medical School, has been appointed to the University readership in physiology tenable at that School.

Nottingham

Dr. R. W. Hepburn, lecturer in moral philosophy in the University of Aberdeen, has been appointed to the chair of philosophy, in succession to Prof. W. J. H. Sprott, who has been appointed to a newly established chair of psychology in the University.

University College of North Staffordshire

The following have been appointed to senior lectureships as from October 1: Dr. D. E. Davies (physics), Dr. J. P. de C. Day (philosophy), Dr. D. S. Lees (economics), Dr. R. McWeeny (theoretical chemistry), A. C. Montefiore (political and moral philosophy), and W. M. Williams (geography).

Sheffield

It is announced that Dr. H. Ruben has been appointed as senior lecturer in charge of the Department of Statistics. The following lectureships are also announced: Dr. D. M. Burley (applied mathematics); Dr. Margaret M. Platts (medicine); H. A. Salmon (physiology).

Announcements

Sir Alexander Fleck, recently chairman of Imperial Chemical Industries, Ltd., has been elected president of the Society of Chemical Industry for 1960/61 in succession to E. J. Solvay, and will be installed at the annual meeting of the Society at Bristol in July.

The Philosophical Society of Washington has announced the election of the following officers for 1960: *President*, E. R. Maxwell; *Vice-presidents*, L. M. McKenzie and R. D. Myers; *Corresponding Secretary*, F. N. Frenkiel; *Recording Secretary*, M. M. Shapiro; and *Treasurer*, L. Slack. Further information concerning the society may be obtained from F. N. Frenkiel, Applied Mathematics Laboratory, David Taylor Model Basin, Washington, 7, D.C.

In written answers to questions in the House of Commons about day-release students in technical education, the Secretary of State for Scotland, Mr. J. Mackay, on February 23 gave the number of boys and girls under eighteen released by their employers in Scotland for part-time education in the year ended July 31, 1958, as 17,934 out of 163,000 estimated employed at the end of May 1958. For England and Wales in 1957–58, Sir David Eccles on February 24 gave the figures 191,889 boys out of 578,000 and 50,636 girls out of 585,000.

In a written reply to a question in the House of Commons on February 24 regarding the professional staff employed by the Atomic Energy Authority, the Minister of Education, Sir David Eccles, as representing the Minister for Science, said that of 4,223 qualified staff, 32 were biologists, 863 chemists, 2 geologists, 239 mathematicians, 990 physicists, 275 metallurgists, 113 chemical engineers, 184 civil and structural engineers, 505 electrical engineers, 1 mining engineer, 1,014 mechanical and other engineers, and 5 held the B.Sc.(Tech).

A symposium on the Formation and Breakdown of Hæmoglobin will be held by the Association of Clinical Biochemists in conjunction with the Biochemical Society on March 26, at the School of Chemistry, University of Leeds. Further information can be obtained from Dr. J. H. Wilkinson, Westminster Medical School, Horseferry Road, London, S.W.1.

The Society of Instrument Technology is arranging a series of one-day conferences. The first is to be held at the Grand Hotel, Bristol, on March 22, and is entitled "The Value of Instruments and Automatic Control to the Smaller Firm". Further information can be obtained from Mr. A. E. Hiden, 13 Chestnut Road, Long Ashton, Bristol.

Norwood Technical College announces that a short course on an introduction to Micro- and Semi-microchemical Methods will commence on April 1. The course will consist of ten weekly lectures and appropriate practical work designed to survey the branches of chemistry in which micro techniques are applied. Further information can be obtained from the Secretary, Norwood Technical College, Knight's Hill, London, S.E.27.

A meeting of the British Food Manufacturing Industries Research Association is to be held in Edinburgh at the McEwan Hall, University of Edinburgh, on March 30. The theme of the meeting will be Science and Food, and besides various speakers an exhibition tracing the history of the canning of foods will be organized. Further information can be obtained from J. B. Pollard, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey.

Under the auspices of the Shell International Petroleum Co., an annual course for science teachers will be held at University College, London, during April 5–8. The opening talk will be given by Lord James of Rusholme, high master of Manchester Grammar School. Three exhibitions concerning oil and related subjects will also be mounted. Further information can be obtained from Mr. J. A. G. Croxson, Shell International Petroleum Co., 1 Kingsway, London, W.C.2.

A third symposium on aspects of agricultural meteorology will be held at Aberystwyth, on March 16, when the subject will be "Upland Climate and Land Usage, with Special Reference to Highland Britain". Memoranda prepared in connexion with the two earlier symposia, on "The Growing Season" and "Shelter Problems", are being reprinted. Further information can be obtained from Mr. James A. Taylor, Department of Geography and Anthropology, University College, Aberystwyth.

PLANNING IN SCIENCE

IN recent years there has been a great deal of discussion in many countries on whether it is possible to plan science, the effect of such planning on science and whether planning does not of necessity threaten the freedom of scientific research or limit the initiative of scientists. These issues were discussed at an international symposium sponsored by the Czecho-Slovak Union of Scientific Workers and held in Prague's Hotel International during September 19–22. Some hundred scientists were present—forty from abroad, from Great Britain, the Soviet Union, France, Brazil, India, Japan, Greece, Poland, Bulgaria, the German Democratic Republic, Rumania, Holland, Denmark, Portugal and North Korea. An observer from Unesco was also present.

The first and fundamental question raised was whether science can, by its nature, be planned at all. The participants in the symposium, without exception, agreed that planning the main tendencies of scientific research and ensuring that they are in harmony with the needs of the country and the development of science is not only possible to-day but also imperative. Naturally, the conditions for the planning of science are very different in a socialist society from those in a capitalist society. However, as pointed out by I. Malek (Czechoslovakia): "Even though scientific development in the capitalist countries has taken its course to date without purposeful and uniform planning, the majority of scientists and public figures responsible for the development of science do not doubt the necessity of planning in some form". P. L. Kapitza (U.S.S.R.) attributed recent spectacular Soviet scientific successes to planning: "The socialist State has great advantages in so far as the planned organization of science is concerned; this is demonstrated by the fact that it was the U.S.S.R. which had the first satellite and also was the first to reach the Moon. We do not think that the quality of our scientists is better than that of scientists of other countries. We are all people and we all know the same, think the same, have the same brains in our heads and our heads are of the same dimensions. But we were able to mobilize the means and the scientists and concentrate them on an important task".

The symposium then went on to discuss whether the planning of science may not become a serious obstacle barring creative research activity. Many of those present admitted that misgivings along these lines could indeed be justified in some circumstances. Care must be taken in formulating the plan, never to forget the individuals who were to carry it out. As M. M. Shemyakin (U.S.S.R.) put it: "Every bit of planning, particularly co-ordination and organization of complex research, must bear in mind the individual creative ability of the worker. Pure administration would not be in place".

Dealing with the potentialities and limitations in the planning of science it was pointed out that in scientific planning the occurrence of unexpected factors, particularly the possibility of basic discoveries in the natural sciences, must be reckoned with. As J. D. Bernal (Great Britain) pointed out: "The difficulties are very largely unforeseeable at the start and it is necessary to change course, to make use of any accidental advantage that crops up, even to abandon the whole attempt and to begin in some other direction. Even then you may not succeed in what you originally aimed at, though maybe instead you will have picked up something unforeseen and even more interesting and useful".

The problem of who should plan and guide scientific research was the subject of lively discussion during which the specific nature of planning in science, requiring special procedure and the widest participation of scientific workers, was stressed. P. L. Kapitza directed attention to the new type of scientific director needed to carry through large scientific projects. Such a director, whose role is creative rather than administrative, may be a very great man even though he himself does not work creatively in science. Interesting light on how scientific administrators are chosen in the U.S.S.R. was given by M. M. Shemyakin: "All heads of laboratories and institutes, all heads of departments at universities are elected every five years by competition in secret voting of the scientific councils. These scientific councils are set up from leading scientists of the given institute and other institutes. The directors of the Academy institutes are also elected for a period of five years from among the most important scientists, also by secret ballot of academicians and corresponding members of the Academy of Sciences of the U.S.S.R. In this way a truly democratic system of directing science is achieved and pre-conditions for control are created".

A great deal of the time of the symposium was devoted to the discussion of the difficult problem of how the results of scientific research can be appraised. It was generally agreed that the appraisal must be done by scientists and certainly not based on financial criteria. But scientists themselves are by no means able always to judge correctly whether a given line of research is likely to lead ultimately to promising results. In the final analysis, J. D. Bernal pointed out, the results of scientific research must be tested in practice. But sometimes, owing to insufficient farsighted appraisal, promising lines of research never get the possibility of practical test. This leads to a great waste of opportunity and a time-lag in putting valuable ideas into practice.

Some of the most interesting contributions to the symposium described how planning operated in practice in a number of countries. In the socialist countries the tendency to proceed from short-term and detailed plans to long-term planning of the main trends and tasks was general. In the U.S.S.R., after a very broad discussion, thirty problems of the natural and social sciences were designated which were recognized as the most timely for the years 1959–65. The planning of science is especially thorough in Czechoslovakia, where a national plan of research including all branches of science and embracing basic and applied research right up to the production stage has been formulated by the Czechoslovak Academy of Sciences. This body is also responsible for securing the financial and material means necessary for carrying it out. Plans for scientific research in Poland, Bulgaria, the German Democratic Republic and North Korea were described.

The only paper describing the organization of science in a Western country was given by E. H. S. Burhop (Great Britain), who pointed out that although an aggregate plan of scientific research in any sense comparable with the plans of the socialist countries does not exist in Great Britain, nevertheless, laboratories working in related fields co-ordinate their work and in the field of basic research this method of free co-operation appears to work satisfactorily. Probably applied research could benefit from more co-ordinated planning, but this met with some difficulty in a social system based on competitive private enterprise.

It would have added to the interest of the discussion if accounts had been available of the organization of science in other Western countries. Nevertheless, the conference was of great value to participants in that it revealed both the similarities and the contrasts between the problems facing scientists in countries having different social systems.

IVAN MALEK

RECENT GEOPHYSICAL WORK IN BRITAIN

AT its meeting on January 27, the Geological Society of London held a symposium on "Geological Implications of Recent Geophysical Work in the British Isles", under the chairmanship of the president, Dr. C. J. Stubblefield. The afternoon session, devoted to the study of igneous rock masses, was opened by Dr. M. H. P. Bott, who outlined the results of recent work on the variation of gravity over granitic intrusions[1-3]. Particular attention was paid to the form of the gravity profiles over two bodies with contrasting features, the Hercynian Bodmin Moor granite and the Caledonian Criffell granodiorite. In the former case a negative anomaly of about 50 milligals could only be interpreted in terms of a large density contrast (thought to be about 0·16 gm./cm.^{3}) between granite and country rock. In contrast, a relatively small negative anomaly of some 20 milligals was recorded over the Criffell granodiorite; the gravity profile could not be straightforwardly interpreted in terms of a uniform density contrast and it was necessary to postulate a major gradational increase of density within the igneous mass towards its south-eastern margin. These conclusions, as well as the space forms deduced for the two intrusions, were not out of harmony with the results of earlier field and petrological studies. It was inferred that both intrusions extended to depths of the order of 11–12 km., more than one third of the normal crustal thickness in continental regions.

Sir Edward Bailey questioned the assumption that there was no significant increase in the density of granites with depth, and quoted examples such as the Ben Nevis complex where the reverse had proved to be the case. Dr. A. H. Cook thought that since it was impossible to estimate densities at depth with accuracy, there was no valid evidence for denying the possibility of granite masses extending down to the Mohorovičić discontinuity. Prof. T. Murphy commented on areas of positive anomaly which flanked the gravity lows over certain Irish granites and stressed the need to consider the regional picture as a whole.

Dr. Bott also discussed the relationship which often appears to exist between the mass deficiency over a granite, calculated from the gravity anomaly, and the excess mass of the topography. This comparison led him to the suggestion that the high topography frequently associated with outcropping granites may sometimes be isostatically compensated, but within the crust rather than at its base.

Dr. D. J. Blundell described some recent investigations carried out to examine the extent to which the magnetic properties of igneous rocks might be of use in geological interpretation. He instanced his own study of the Lundy dyke swarm[4], in the course of which he was able to show that the mean direction of magnetization of the intrusions agreed with that of Tertiary lava flows. Another example cited was the work of Dr. D. Armstrong on some minor intrusives of Ayrshire[5]. This showed that the kylites and crinanites formed two separate suites with distinct polarization directions, the former corresponding to the known Permian direction, the latter consistent with a Tertiary age.

Palæomagnetic work on the Newer Basic Intrusions of Aberdeenshire gave rise to some discussion. Dr. Blundell and Prof. H. H. Read[6] interpreted the results as indicating that the Huntley, Insch and Belhelvie gabbros had been intruded in approximately their present attitudes and had not suffered subsequent deformation, a conclusion carrying important structural and petrogenetic implications. Prof. F. H. Stewart and Prof. R. M. Shackleton found it difficult to accept the palæomagnetic evidence as conclusive, pointing out the need for more detailed study of the geology of the Insch mass and of the effect of such features as the ore mineralogy and the thermal history on the magnetic behaviour of the rocks.

Dr. D. W. Powell quoted difference in magnetic dip between two Scottish Lower Carboniferous basalt lava sequences, both of which contained reversals, indicating polar movement during the period. He commented also on the effect of compression on rock magnetization as shown by laboratory experiments.

Mr. S. H. U. Bowie described the results of an airborne radiometric survey of Devon and Cornwall carried out for the Atomic Energy Division of the Geological Survey[7]. This had disclosed a relatively even level of radioactivity over the different granite masses, though individual members such as the Castle an Dinas phase of the Land's End granite, as well as kaolinized areas in general, were markedly low in activity. Acid igneous rocks gave much higher values than basic varieties and there was a general tendency for radioactivity to rise in the late differentiates of granites. On Lundy Island, however, Dr. A. T. J. Dollar had found the earlier granite to be the most highly radioactive member of the complex.

The evening session dealt with the study of sedimentary basins and their sub-surface geology. The results of seismic work in the Carboniferous basin of the East Midlands and the Hampshire Mesozoic and Tertiary basin were outlined by Mr. R. G. W. Brunston and Mr. N. G. O'Halloran. With increasing knowledge of the general structural picture and the need for detailed exploration and study of individual structures in the course of the search for oil within

these basins, gravity and seismic refraction surveys were now being superseded by seismic reflexion work. In the East Midlands many pre-Permian folds had been discovered by this method and afterwards confirmed by drilling. A smaller number of pre-Albian folds had been found in Hampshire and Dorset and the margins of the basin more accurately delimited.

Dr. W. Bullerwell described the results of aeromagnetic surveys over central, eastern and southern England carried out under contract for the Geological Survey. Regions of positive anomaly were located in the northern parts of Leicestershire and Northamptonshire, in the area surrounding The Wash, along a magnetic 'ridge' extending roughly from Reading to Birmingham and in the English Channel south-east and south-west of the Isle of Wight. Comparison of the magnetic map with the gravity map of the same area, based upon data provided by the Geological Survey, University of Cambridge and the British Petroleum Co., revealed many regions in which gravitational and magnetic anomalies showed clear positional relationships, either directly or inversely correlated as regards amplitude. The general picture of the sub-Mesozoic surface of central England was suggestive of a block pattern in which the trends of such familiar structural lines as the Lickey, Nuneaton and Charnwood 'axes' were discernible.

The combined gravity and magnetic data, supported by the evidence of deep boreholes, afforded a suggestion of a sub-Mesozoic surface of acid igneous material at relatively shallow depth between Market Harborough and Bedford; and of a north-westerly trending geosyncline filled with Lower Palæozoic sediments beneath southern East Anglia, an interpretation in harmony with views previously expressed by Sir Edward Bullard and others after seismic investigations in this area[5].

Recent research, outlined by Prof. W. B. R. King and Prof. W. F. Whittard, supplemented previous knowledge of the geological history of the English Channel and Western Approaches[9–11]. In the central and eastern Channel, seismic work had proved high-velocity rocks at much less depth than anticipated. It appeared that the Paris Plage Palæozoic ridge was of greater extent than formerly thought and must virtually have separated the Hampshire and Paris basins. Probably the Trias was absent over this ridge and the Jurassic succession much thinner than that in the Portsdown and Henfield borings to the north and the Pays de Bray to the south. In the Western Approaches, Prof. Whittard inferred a series of tongue-shaped outcrops ranging from Upper Cretaceous to late Tertiary, with the younger formations appearing progressively towards the south-west. On the continental slope indurated blocks of chalky material containing Recent, or at earliest Pleistocene, Foraminifera had been dredged from a depth of 500 fm.

Among other contributions dealing with marine geophysics was one from Dr. R. F. King describing seismic, gravity and magnetic surveys in the northern part of Cardigan Bay, supplementing previous work by Dr. D. W. Powell[12]. The density and seismic velocity of the upper layers suggested the presence of clays of late Mesozoic or Tertiary age. Mr. G. Armstrong described the use of the sub-bottom depth recorder (or 'sparker'), essentially a broad-band, high-powered, echo-sounder, using as a sound source either an electric spark or a small explosion. With this instrument the National Coal Board had obtained a reasonably comprehensive picture of the fault pattern, disposition of strata and distribution of igneous dykes off the coast of Northumberland and in the Firth of Forth. An oblique echo-sounder system which recorded reflexions from the sea-bed from a low-angle beam had been successfully applied for geological purposes in Weymouth Bay by Dr. D. J. Donovan and Mr. A. H. Stride.

A feature of the symposium was the variety of the techniques discussed and the complementary nature of the geological evidence obtained by different methods. It was noteworthy that many of these (aeromagnetic, aeroradiometric, 'sparker', 'oblique asdic') employed continuous recording along a chosen profile. Increasing use of such methods is an important trend in applied geophysics at the present time and their advantages both in speed of operation and for interpretation were pointed out towards the close of the evening by Dr. Bullerwell.

J. H. Taylor

[1] Bott, M. H. P., *Geol. Mag.*, **90**, 257 (1953).
[2] Bott, M. H. P., *Quart. J. Geol. Soc.*, **112**, 45 (1956).
[3] Bott, M. H. P., Day, A. A., and Masson-Smith, D., *Phil. Trans. Roy. Soc.*, A, **251**, 161 (1958).
[4] Blundell, D. J., *Geol. Mag.*, **94**, 187 (1957).
[5] Armstrong, D., *Nature*, **180**, 1277 (1957).
[6] Blundell, D. J., and Read, H. H., *Proc. Geol. Assoc.*, **69**, 191 (1959 for 1958).
[7] Bowie, S. H. U., Proc. 2nd Int. Conf. Peaceful Uses Atomic Energy, Geneva, **2**, 787 (1958).
[8] Bullard, E. C., Gaskell, T. F., Harland, W. B., and Kerr-Grant, C., *Phil. Trans. Roy. Soc.*, A, **239**, 29 (1940).
[9] Hill, M. N., and King, W. B. R., *Quart. J. Geol. Soc.*, **109**, 1 (1953).
[10] King, W. B. R., *Quart. J. Geol. Soc.*, **110**, 77 (1954).
[11] Day, A. A., Hill, M. N., Laughton, A. S., and Swallow, J. C., *Quart. J. Geol. Soc.*, **112**, 15 (1956).
[12] Powell, D. W., *Quart. J. Geol. Soc.*, **111**, 375 (1956 for 1955).

EVOLUTION OF ARBORVIRUS DISEASES

THE arboviruses (*arthropod-borne* viruses) are those animal viruses which multiply in the tissues of the arthropod host and are transmitted in its saliva. The purposes of a symposium which had been arranged by the Royal Society of Tropical Medicine and Hygiene, and was held in London on January 21, were as follow: (1) to try to fit the arboviruses into current evolutionary theory; (2) to review current knowledge of arboviruses and suggest profitable lines of thought and investigation; (3) to interest workers in related disciplines, notably zoologists.

The first two papers dealt mainly with the origin and long-term evolution, the third mainly with evolution observable in our own time.

Prof. C. D. Darlington discussed the relationship between heredity and infection in plant, insect and animal viruses. Human viruses are particularly fruitful for study because while viruses are the most rapidly evolving of parasites, man is the most rapidly evolving, most varied and most widely distributed of higher organisms. Because viruses are wholly parasitic we have to consider the relations of harm or benefit between hosts and parasite. At the virus-

level, the fundamental distinction in the basis of propagation is between the nucleic acids involved. In plants the two extreme forms of ribonucleic acid particle are the purely hereditary plasmagene of male fertility and the purely infectious particle of tobacco mosaic virus. Particles which are artificially transmissible by grafting, but not spread by natural infection, may be called proviruses, while viruses which apparently appear spontaneously or by chemical or physical treatment may be called new viruses. When vectors transplant viruses to new species the behaviour of the virus may change greatly, and when a vectored virus is artificially transmitted in series it may lose the capacity to infect the vector. A virus can become a harmless nucleoprotein (become latent): or something hereditarily transmitted in the cytoplasm or generated by the cell in development may become infectious. Latent viruses which resemble the plasmagene in behaviour may arise from active viruses either in the capital host or the vector and may or may not be transmitted through the egg. Further evolution may lead to loss of infectiousness, as with Kappa in *Paramecium*, or the hereditary vector stage may be lost and only direct infections between capital hosts remain. Phages have evolved on a basis of alternating heredity and infection and it is possible that some viruses of higher organisms may also have done so. Heredity and infection seem, however, to be strict alternatives, for the viruses of higher organisms and hereditary particles are likely to persist only when they confer a benefit on the host. On the other hand, infectious particles owe their maintenance to the benefit they extract from the host, and harm to the host is relatively unimportant. The selection pressures are, therefore, opposed and when a hereditary particle becomes infectious they are switched.

Mr. P. F. Mattingly presented the view that the evolution of arboviruses has depended very largely on the ecology of their arthropod and vertebrate hosts. Despite their isolation in host tissues, each individual virus may be credited with an ecology of its own, just like other organisms. To appreciate this, secondary accretions such as adventitious transfer to man and occurrences in atypical hosts or vectors must be disregarded. When it was introduced into the New World, yellow fever rapidly found an ecological milieu very closely resembling that from which it had been liberated in Africa. The need seems to have been primarily for arboreal primate hosts. *Haemagogus* were available, in place of forest *Stegomyia*, and adaptation to these may have been relatively easy. Similarly the principal vectors of Wesselsbron virus in the low and high veldt (*Neomelaniconion* and *Ochlerotatus* respectively) are taxonomically distinct, but phenologically closely similar.

The present-day requirements for the establishment of a mosquito-borne virus appear to be the coexistence of warm-blooded vertebrates, angiospermous plants and blood-sucking mosquitoes. The two former are known to have evolved more or less together during the latter part of the Mesozoic and the viruses and their mosquito vectors probably evolved with them. The necessity of fitting into a complex ecological system may have slowed the rate of evolution compared with some other viruses. The fact that primate malaria can be transmitted only by anopheline mosquitoes while bird malaria is readily transmitted by culicines in the laboratory and, presumably, also in Nature, suggests that the anophelines originated as mammal feeders and the culicines, at least in part, as a bird-feeding group. The few known cases of virus transmission by anophelines, other than *Kerteszia*, appear to be adventitious or secondary. Basic maintenance cycles involve culicines only, except possibly in the subgenus *Kerteszia* which in some ways are more culicine than anopheline in their ecology. These facts suggest that the primitive mosquito-borne viruses were bird rather than mammal viruses. *Aëdes*-borne viruses are confined to the tropics. In the subtropics they are replaced by *Culex*-borne viruses and these in turn are replaced by tick-borne ones in the northern steppes. The explanation seems to be that *Aëdes* hibernate and æstivate in the egg, *Culex* as adults. In the relatively severe conditions prevailing in the subtropics hibernating adult mosquitoes appear to be necessary for the maintenance of virus. Farther north, only the resistant egg will serve for hibernation and *Aëdes* become the dominant mosquitoes. But in the absence of trans-ovarian transmission these are unsuited to the maintenance of virus, and mosquito-borne viruses are replaced by tick-borne ones. On the assumption that West Nile is the most primitive of the group *B* viruses, a picture can be drawn of invasion from sub-tropical swamp habitats to the northern steppes on one hand, and to a variety of tropical habitats on the other, with transfer to ticks and to *Aëdes* respectively. A simpler and more coherent picture would result from the assumption that the Russian spring–summer viruses were the most primitive, and the arguments previously adduced are worth reconsideration on this basis.

Dr. C. E. Gordon Smith concentrated mainly on the effects of intentional or unintentional human interference on the short-term evolution of arboviruses. About a third of the known arboviruses cause human disease ranging from severe encephalitis to mild fever—the remainder are still in search of a disease, but there are also many human febrile diseases which may be in search of a virus. Arboviruses are most numerous in the tropics and become progressively fewer as we go north or south to sub-tropics and temperate zones.

The viruses are usually maintained in their wild reservoirs without overt signs, and infections of man, and epidemics in man, spill over from these reservoirs under suitable conditions. Such infections are, however, without significance to the continued survival of the virus.

When a virus is in equilibrium with its hosts and environment (endemic or enzootic), the strain present is the one whose characteristics most perfectly fit the particular ecological complex. Under such conditions a virus mutant is very unlikely to be better adapted for survival than the parent form and is unlikely to succeed. Any change in the number or species of hosts or in the environment, however, provides conditions which may favour a new mutant, which may, by chance, be more or less virulent for man than the parent form. The sudden appearance of Kyasanur Forest disease in western India in 1955, and of a hæmorrhagic 'dengue' in the Philippines and Thailand in 1956, suggest that changes of these sorts must have occurred.

Among man's major activities the clearance of forest and introduction of new hosts to cleared areas are of outstanding importance. Unsuspected viruses may be liberated to establish themselves elsewhere. Russian spring–summer encephalitis became an important disease in Russia only when the taiga forests were cleared and settled.

Irrigation and subsequent agriculture in California brought changes in the flora and fauna which were responsible for epidemics of encephalitis, and increased agricultural activity in Tongaland appears to have led to greatly increased arborvirus activity there.

With the widespread use of insecticides, resistant arthropod strains emerge, and it is unlikely that they do not also differ from the parent form in other respects, such as vector efficiency for arborviruses. Arthropods do not appear to be adversely affected by arborvirus infection and it is not known whether their defence mechanisms interact with the viruses. In vertebrates the specific defence is antibody formation, but there are also the non-specific processes of inflammation and fever. Inflamed tissue is acid, thus a strain of virus with greater resistance to acid and which multiples better over 100° F. may prevail. In the arthropod, the length of the extrinsic incubation period is dependent upon temperature; thus, on the other hand, viruses which multiply better at, say, 70° F., might have an advantage.

A strain of virus capable of remaining latent in vertebrates for long periods might be maintained through periods when arthropods are not sufficiently numerous. Such latent infections with western equine virus have been demonstrated in birds and the stress of migration or ovulation might permit the virus to circulate when mosquito populations are becoming active in a temperate climate.

Opening the discussion, Dr. C. H. Andrewes suggested that there are two kinds of evolution to consider: the short-term with changes such as have been best seen in influenza, and the long-term, in which viruses as a whole may be very old and relatively stable. Viruses have a unique form of replication, which does not suggest that they are derived from host components. The ticks being probably more primitive than the mosquitoes might be more likely to have been the original hosts.

Several other speakers suggested pointers to the ticks as original hosts. Prof. D. S. Bertram pointed out that the one-layer covering of tick eggs may make them more susceptible to transovarial infection than mosquito eggs. Dr. M. G. R. Varma said that it appeared that while mosquito-borne viruses could multiply in ticks, tick viruses could not in mosquitoes. Dr. P. B. Stones suggested that if mosquitoes were the origin, then viruses ought to have been isolated from male mosquitoes: he reported an isolation of Semliki forest virus from a male *Eretmapodites* in Lagos.

Dr. J. S. Porterfield suggested that Semliki Forest virus may be the most primitive member of group *A* of arborviruses—a number of very closely related forms have now been isolated in Africa. He thought that Israel turkey virus may be a recent derivative of West Nile virus possibly resulting from man-made ecological changes.

On an analogy with transduction in bacteriophage, Dr. P. H. A. Sneath suggested that viruses are not true parasites, but may be partially derived from the reproductive mechanisms of their hosts.

C. E. Gordon Smith

RED DEER AND VEGETATION IN NEW ZEALAND

An expedition visited Lake Monk in the Cameron Mountains of Southern Fiordland, New Zealand, during March 26–April 5, 1957, to study the interrelations of birds and mammals, particularly deer, with the vegetation (New Zealand Department of Scientific and Industrial Research, Bull. 135).

The vegetation was classed broadly into forest, scrubland, grassland and bogs. Mice and stoats are the only small animals in the area; the former are scarce and the latter are probably more widely distributed; from an examination of droppings they appear to be feeding mainly on birds. Red deer (*Cervus elaphus*) are thought to have reached the area about 1920, and to have built up a peak population between 1943 and 1950. They have since declined in numbers and are probably still declining. The population was composed of 50 stags to 35 fawns to 100 hinds, which are unusually low ratios; the deer were all in very poor condition.

The amount of grazing on the fern *Polysticum vestitum* was strongly correlated with the density of deer fæces, and could be used to provide an alternative index to deer abundance.

Counts of deer fæces showed that the animals in this area were spending more time in the forest than in the open grassland, although their preferred food at the time of year studied is grasses and forbs, but storms probably prevent the deer from spending as much time in the open as they would otherwise do. In the forest the beech seedlings are heavily grazed, but once established they continue to grow slowly. The saplings are hedged by deer; but the full amount of each year's growth is not removed and eventually a shoot grows above the browse-level There are sufficient pole-stage beech to replace the present generation of mature trees, and enough of the younger stage to ensure the continuance of the forest, although possibly with a lower density of trees.

The second-tier species in the forest, such as *Nothopanux colensoi*, *Griselinia littoralis*, and *Coprosma foetidissima*, have little resistance to browsing and the young stages of these species are only found in places inaccessible to deer. In the subalpine scrub there has been a widespread destruction of *Olearia colensoi*: grassland areas have been heavily used but show signs of recovery. On boggy areas the trampling of hooves has bared the ground around wallow holes.

The investigators concluded that red deer provide the dominant animal influence on the vegetation; but it is an influence superimposed on long-term trends due to climate and on short-term successional changes. The composition of the vegetation has been and is being altered by them in such a way that there is a marked increase in unpalatable plants, and a corresponding decrease in palatable plants. The resulting environment is relatively unfavourable to deer. In spite of these changes in the composition of the vegetation the forest, as a protective forest, is in no danger of being eliminated by deer; the total vegetative cover is not decreasing, and soil erosion in the area is negligible.

In the light of these findings an attempt is being made to define and clarify the wild-life problems arising from the use of this area as a national park.

THE LONG ASHTON RESEARCH STATION

THE volume of work reported in Long Ashton's annual report* is now so large that some selection is necessary to keep a review within manageable proportions.

Growth of apple fruits is dependent on the presence of seeds, but D. L. Abbott finds that this is so only during seven weeks after petal fall. C. Bould worked on the nutritional aspects of fruit production and found that yield and growth responses of Royal Sovereign strawberries in relation to nitrogen, phosphate and potash were those to be expected from leaf analyses of non-manured plants at the fruiting stage. This should provide a useful practical approach to the vital question of soil nutrient needs. Pollination is a necessary factor in fruit production, and it is surprising that relatively little is known about the distance over which a pollinator variety is effective. R. R. Williams found it to be about 90 ft. in one orchard, but only 50 ft. in another. A survey by A. I. Campbell and R. R. Williams of eighty orchards in Hereford and Somerset shows that difficulties encountered in headworking cider apple trees are in part due to apple virus diseases. It is sometimes necessary to clear old tree-stumps from land; E. W. Hobbis and J. S. Coles have investigated various possible rotting agents—aqua regia, caustic soda, bleaching powder, and a nitrogen–phosphate fertilizer. Only the last material showed any effect after four years. Workers in the pomology section also discuss the technique of measuring the assimilation of radioactive carbon by leaves, a survey and descriptions of perry pear varieties, and the forecasting of harvest date in blackcurrant varieties from their flowering dates.

* Annual Report of the Agricultural and Horticultural Research Station (The National Fruit and Cider Institute), Long Ashton, Bristol, 1958. Pp. 190+18 plates. (From the Station, 1959.) 15s. post paid.

A series of papers on spray application problems gives methods for the determination of spray residues —of gamma-BHC (J. A. Pickard), of microgram quantities of dieldrin (E. J. Skerrett and E. A. Baker), and of rotenone in *Lonchocarpus* (D. V. Richmond). A mercury content of 1 p.p.m. was found in coffee berries on trees which had received thirteen applications of mercury fungicide (J. A. Pickard and J. T. Martin). The plant cuticle is the immediate receptor for spray fluids, and J. T. Martin reviews current work on its nature and composition in several fruits and vegetable crops. Uptake of a fungicide (DMTD) by fungal spores (E. Somers), and the metabolism of certain aromatic compounds by fungi (D. Woodcock and R. J. W. Byrde) are also discussed. There has been quite a revolution in outlook on methods for spray application, and T. E. Cobbald briefly describes a prototype low-pressure twin-fluid atomizer. Fluorescent materials as tracers in spray liquids, and the vibrating jet method for the determination of dynamic surface tensions are two methods of assessing spray performance discussed in the report. Considerations of actual disease are mainly concerned with *Gloeosporium* fruit rot, and entomology is represented by a paper on seasonal distribution of four species of leaf hoppers on apples. An interesting result of importance overseas is the finding of the fungus *Trachysphaera fructigena* on Jamaican bananas. The infection appears to have occurred in ripening rooms, from fruit from another source.

There are papers on cider and fruit juices and on domestic fruit preservation, as in previous years, and a list of 46 publications during 1958. It is announced in the introduction that the Agricultural Research Council's Unit of Plant Nutrition at Long Ashton was disbanded in September 1959. Members of its staff have, however, been absorbed into the work of the Station.

JOHN GRAINGER

INFLUENCE OF NITROGEN-CONTAINING BASES ON THE RATE OF THE ELECTRODE PROCESSES AT THE DROPPING MERCURY ELECTRODE

By MRS. V. VOLKOVÁ

Polarographic Institute, Czechoslovak Academy of Sciences, Prague

A SPECIFIC effect of gelatin, amines, *p*-aminobenzoic acid esters of amino-alcohols and nitrogen-containing heterocyclic compounds such as various pyridine derivatives, alkaloids, etc., has been found[1,2] during the polarographic reduction of many organic compounds.

In respect of the polarographic waves, the effect on the reduction of substances capable of dissociation was of two kinds: on one hand, an influence on the height of the first wave, on the other hand, a considerable shift of the second wave to more positive potentials, often by several hundreds of millivolts.

The first case was observed chiefly with very weak acids, containing a heterocyclic nitrogen atom such as pyridine carboxylic acids, quinaldinic acid and oxyquinolines, and with corresponding aldoximes, further with α-Br-carboxylic acids, phthalic acid and benzaldehyde, the shift of the second wave being found with many reducible weak acids to such an extent that only a single wave was visible.

The effect on the first wave was originally explained by a reaction controlled by the recombination of the anion with the nitrogen-containing cation as proton-donor[1]. The recombination-rate constants thus obtained were of the order 10^9–10^{10} except with atropine and some other alkaloids, where considerably larger values were obtained which depended on the concentration of the nitrogen compound.

Since the above-mentioned catalysts are substances of strongly adsorptive character, attention has been directed to the study of the role that this property takes in the problem studied. The conception of the

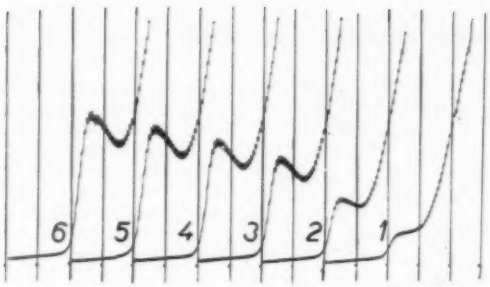


Fig. 1. Effect of amino-pyridines on the first wave of 10^{-3} *M* isonicotinic acid; borate buffer *p*H 10; concentration of 2-amino-pyridine: (1) 0, (2) $0{\cdot}5 \times 10^{-2}$ *M*, (3) $1{\cdot}5 \times 10^{-2}$ *M*, (4) 2×10^{-2} *M*, (5) $2{\cdot}5 \times 10^{-2}$ *M*, (6) 3×10^{-2} *M*; each curve starts at $-1{\cdot}0$ V. (vs. S.C.E., 0·2 V./absc.)

adsorption of the catalyst was supported in this case by the fact that the catalytic influence tended towards a limit with respect to the concentration of the catalyst. The influence of the adsorption of cations has been observed before in the polarographic reduction of inorganic anions; recently this case was thoroughly discussed by Frumkin[3]. Evidence for the adsorption occurring in our case as an accumulation of the catalyst at the electrode surface has been obtained from the study of *i*–*t* curves. When alkaloids, which have a large adsorption coefficient, were used as catalysts the effect of acceleration of the electrode process with time (a distinct inflexion on *i*–*t* curves) occurred at potentials where the mean current represented less than a quarter of the limiting current, but in the presence of pyridine derivatives and gelatin the *i*–*t* curves exhibited always the monotonous kinetic course.

The shape of *i*–*t* curves for different concentrations of the alkaloid (see Fig. 2 with phenylglyoxylic acid in the presence of atropine) corresponds to the concept of acceleration of the electrode process by the adsorption of a polarographically inactive substance with a large adsorption coefficient on the electrode surface. Because of the large adsorption coefficient, a certain period of time has to pass before the quantity of surface-active substance necessary for the saturation of the electrode surface is transferred to the electrode by diffusion.

Due to this increase of the surface concentration of the catalyst at the electrode the rate of the electrode process is also gradually increased until the limit concentration on the electrode is reached. The influence of the adsorption of an electrode-inactive substance with a large adsorption coefficient on the electrode processes of depolarizers has been theoretically solved by Weber, Koutecký and Koryta[4,5]. For the instantaneous current *i* at the beginning of the rising portion of the polarographic wave for periods of time smaller than ϑ (the time when the surface coverage has been attained) and for cases in which the rate constant at the occupied surface k_1 is distinctly larger than that at the free surface k_0 the following relation, $i \sim t^{1/6}$, holds evidently only in the case when the effective rate constant k_{ef} is a linear function of θ, the degree of the coverage of the electrode, and k_1 does not change with this degree of the coverage of the electrode. The relations between these quantities are as follows[6]:

$$\theta = \frac{\Gamma}{\Gamma_k} = \left(\frac{t}{\vartheta}\right)^{1/3}; \quad \vartheta = 1{\cdot}85 \times 10^6 \times \Gamma_k^2 \times c_A^{-2} D_A^{-1}$$

where Γ and Γ_k are the instantaneous and maximal concentrations of the catalyst adsorbed at the electrode surface, c_A the concentration of the surface-active catalyst in the solution, and D_A its diffusion coefficient.

The *i*–*t* curves at the beginning of the rising portion of the wave of the anion of phenylglyoxylic acid in the presence of alkaloids represent the most suitable example for the relations mentioned above. Although the exponent for $t < \vartheta$ was larger than 7/6 (up to 1·6) the dependence of ϑ on c_A^{-2} was found to be linear. Putting $D_A = 6{\cdot}4 \times 10^{-6}$ cm.²/sec. the area occupied by one molecule of atropine has been calculated as 93 Å.². The higher value of the exponent can be explained by the assumption that the adsorption is a kinetic process, or by the change of k_1 during the drop time with the change of θ or by supposing that for other reasons (the change in the value of the potential ψ_1) k_{ef} is not a linear function of θ. This will be the object of further investigations.

In the case of substances with a small adsorption coefficient the adsorption equilibrium is strongly shifted in favour of the non-absorbed form, so that Γ remains constant during the drop time and if the equilibrium is established very rapidly Γ can be expressed by any adsorption isotherm. Such a case can be solved for mean currents as in a normal slow electrode reaction[7]. If k_{ef} is a linear function of θ for suitably small concentrations for which θ is

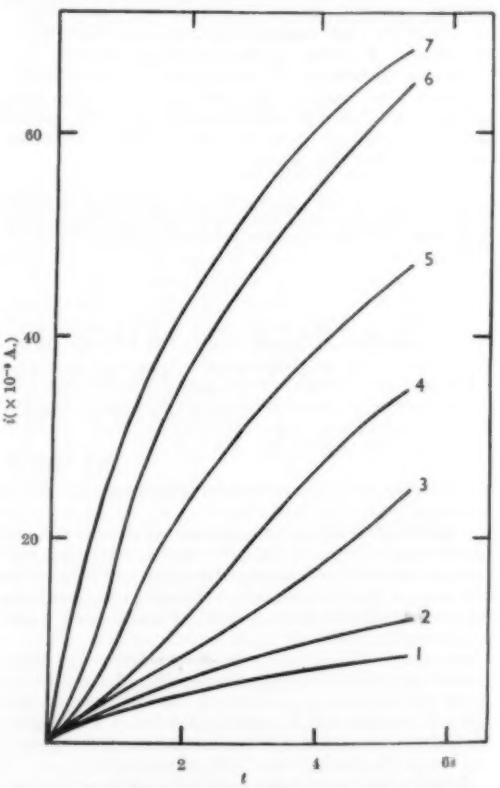


Fig. 2. Effect of alkaloids on *i*–*t* curves of 4×10^{-3} *M* phenylglyoxylic acid (as depolarizer); borate buffer *p*H 8·6. $E = -1{\cdot}04$ V. (vs. S.C.E.); concentrations of atropine: (1) 0, (2) $0{\cdot}4 \times 10^{-5}$ *M*, (3) $2{\cdot}3 \times 10^{-5}$ *M*, (4) 5×10^{-5} *M*, (5) $8{\cdot}3 \times 10^{-5}$ *M*, (6) $13{\cdot}3 \times 10^{-5}$ *M*, (7) $23{\cdot}3 \times 10^{-5}$ *M*

also a linear function of the concentration of the catalyst in the solution and at a constant pH, then: $\frac{i}{i_d - i} \sim C_{kat.}$ A good agreement of experiments with this relation has been found for some derivatives of pyridine.

A plausible explanation of the acceleration of the electrode process is as follows: since in all investigated cases the adsorption occurred at potentials at which the electrode has a negative charge, it can be assumed that the surface-active substances are adsorbed chiefly as cations. The circumstance that a very distinct acceleration of the electrode process occurs if anions are reduced or undissociated substances with the possibility of forming zwitterions such as *iso*nicotinic acid, hydroxyquinolines, pyridinealdoximes, etc., would support the assumption that a closer approach of the depolarizer to the electrode is made possible, perhaps because of a tendency to form an adduct. The distinct increase of the current of the second wave of phthalic acid which is ascribed to the reduction of the undissociated acid[8] could be explained by the assumption that the acid exists in the form with separated charges the electrode reaction of which is catalysed. The idea of thus influencing the reduction is supported also by the finding that in the reduction of a particle with a positive charge such as in the reduction of the cation of phthalic acid the presence of a surface-active substance as a cation causes a lowering of the current. There is no tendency towards the formation of an adduct in this case; on the contrary, the presence of the cation at the electrode surface causes a repulsion of the depolarizer with a positive charge and therefore a retardation of the electrode process must take place.

The details will be published elsewhere.

[1] Volková, V., Czechoslov. Hungar. Polarograph. Conference, Prague (July 1958).

[2] Volková, V., Second International Polarographic Congress, Cambridge (August 1959).

[3] Frumkin, A. N., *Trans. Faraday Soc.*, **55**, 156 (1959).

[4] Weber, J., Koutecký, J., and Koryta, J., *Z. Elektrochemie*, **63**, 583 (1959).

[5] Weber, J., Second International Polarographic Congress, Cambridge (August 1959).

[6] Koryta, J., *Chem. Listy*, **47**, 340 (1953); *Coll. Czech. Chem. Comm.*, **18**, 206 (1953).

[7] Koutecký, J., *Chem. Listy*, **47**, 323 (1953); *Coll. Czech. Chem. Comm.*, **18**, 597 (1953).

[8] Ryvolová, A., and Hanuš, V., *Chem. Listy*, **50**, 46 (1956); *Coll. Czech. Chem. Comm.*, **21**, 853 (1956).

SOME SIMILARITIES AND DISSIMILARITIES IN THE STRUCTURE OF THE SKIN AMONG THE MEMBERS OF THE SUBORDERS ODONTOCETI AND MYSTACOCETI (CETACEA)

By Dr. W. SOKOLOV

Department of Vertebrate Zoology, University of Moscow

THE members of two cetacean suborders, while possessing a number of similar features, show at the same time some essential differences. Obviously, the same relations must exist in the structure of their skin as well.

Up to now no attempts have been made to compare the structure of the skin in those suborders, although the cutaneous covering of some cetacean species has been studied by several investigators[1].

As material for the work reported here, samples of the skin from the following species of Cetacea were used: *Eubalaena glacialis* Bonaterre (obtained in 1955 by permission of the Minister of Fisheries *N*0113/2010, dated March 3, 1955), *Balaenoptera physalus* L., *B. borealis* Lesson, *B. acutorostrata* Lacépède, *Physeter catoden* L., *Berardius bairdii* Steineger, *Delphinus delphis* L., *Lagenorhinchus obliquidens* Gill and *Orcinus orca* L.

Most samples were collected during the expedition sent by the Institute of Oceanography of the Academy of Sciences of the U.S.S.R. (head of the expedition, S. Klumov).

Samples taken across the whole skin up to subcutaneous muscles were embedded in 'Celloidin'. Sections made by Christeller's method were stained with hæmotoxylin-eosin, after Van-Gieson and with resorcin-fuchsin.

Peculiarities of the cetacean skin common to all members of that order will be considered first. The cutaneous covering of Cetacea differs from that of other mammals by the absence of a continuous pelage and skin glands. Their skin consists of the following layers: the epidermis (Figs. *A*1, *B*1), the dermis (Figs. *A*2, *B*2) and the subcutaneous fat tissue (Figs. *A*3, *B*3). The limit between two latter layers is slightly marked. The epidermis, though reaching considerable thickness, consists of only three layers: the outer one, called conditionally the corneous layer (cornification does not take place in it and its cells retain nuclei) (Figs *A*4, *B*4); the underlying stratum spinosum or prickle cell layer (Figs. *A*5, *B*5); and the deepest basal layer (Figs. *A*6, *B*6). On the inner surface of the epidermis numerous alveoli are distributed, in which dermal papillæ rise vertically upwards (Figs. *A*7, *B*7). The epidermis may be pigmented, the pigment in the form of finest granules being disposed in the epidermal cells and localized above the cell nucleus. At the limit between the epidermis and dermis melanophores sometimes occur.

The dermis of Cetacea consists of dermal papillæ (Figs. *A*7, *B*7) and of a sub-papillary layer (Figs. *A*10, *B*10) in which a non-adipose layer (Fig. *B*11) adjoining the epidermis may occasionally be seen. The dermal papillæ are formed of thin bundles of collagenic fibres rising vertically upwards along the long axis of the papilla, and of a few elastic fibres running in the same direction. Small blood vessels rise to the top of the papilla (Fig. *A*12). No fat cells are present in the dermal papillæ. In the subpapillary layer of the dermis bundles of collagenic fibres (Figs. *A*13, *B*13) are situated at various angles to the surface of the skin, forming a more or less dense network. Elastic fibres may be numerous. The thickness of the latter as well as that of the bundles of collagenic fibres is considerably greater than in papillæ. Fat cells (Figs. *A*14, *B*14) are disposed either immediately at the epidermis itself or

at some distance from it, in which case a non-adipose layer (Fig. *B*11) with a very compact network of bundles of collagenic fibres will be formed. The dermis passes gradually into a subcutaneous fat tissue (Figs. *A*3, *B*3) in which collagenic fibre bundles (Fig. *A*13, *B*13) are disposed far from each other, the whole space between them being filled up with enormous accumulations of fat cells (Figs. *A*14, *B*14). With their removal from the epidermis the bundles of collagenic fibres become thicker and the fat cells grow larger, decreasing only in size near subcutaneous muscles. Elastic fibres and large blood vessels are but few in the subcutaneous tissue. A vast number of capillaries pass between the fat cells.

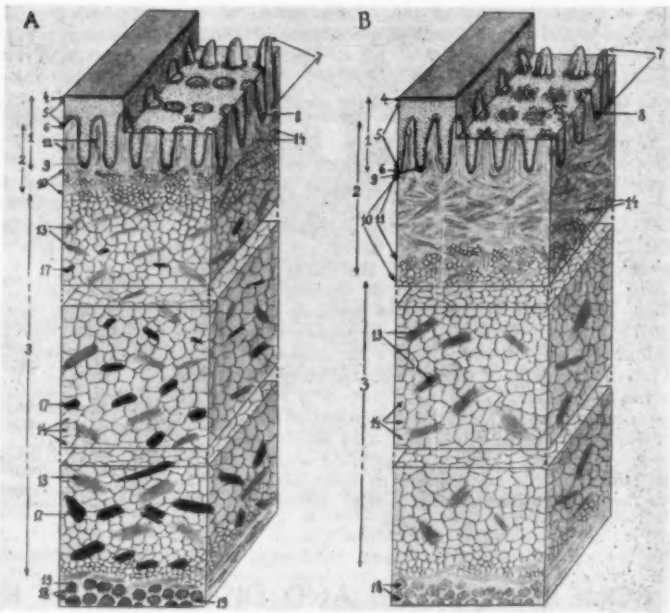
In describing differences of the skin in two suborders of the order Cetacea, two families, Balænopteridæ and Balænidæ, among Mystacoceti, have to be considered separately because the skin of the latter combines characters of both the Odontocete and Balænopteridæ. The skin of *Eschrichtius gibbosus*, family Eschrichtiidæ, has not been studied.

On a tangential section of epidermis the epidermal alveoli of Balænopteridæ and of the surface-swimming Odontoceti have the form of an oval (Fig. *A*16), the long axis of which is directed along the animal's body. In deep-diving Odontoceti (*P. catodon* and *B. bairdii*) and in *E. glacialis* the form of the alveolus diameter is rounded almost over all their height (Fig. *B*). Epidermal septa delimiting the alveoli on a tangential section on their long sides (in parallel with the long side of alveolar oval) and which are called longitudinal septa, in Mystacoceti (Fig. *A*8) penetrate deeper into the subpapillary layer of the dermis than transversal septa (Fig. *A*9). But in most Odontoceti their height is nearly equal (Figs. *B*8, *B*9). In the epidermis of Odontoceti projections from epidermal septa (Fig. *B*15) grow into the tissue of dermal papillæ (Fig. *B*7). In *D. delphis* and *L. obliquidens* projections are absent, but there exist separate projecting epidermal cells. In Balænopteridæ the epidermal septa have no projections except small ones found in *B. acutorostrata*. In *E. glacialis* projections reach a considerable size. The functional significance of those projections consists in increasing the strength of cohesion between epidermis and dermis and in enlarging the area of the producing surface of epidermis, namely, of its basal layer.

In more cold-loving Balænopteridæ the subpapillary layer of dermis is nearly absent, being replaced by a subcutaneous fat tissue (Fig. *A*3). Fat cells are located immediately under the epidermis, creating thereby a reliable thermoisolation. In most Odontoceti the subpapillary layer of dermis is well marked (Fig. *B*10), a non-adipose layer being usually present therein (Fig. *B*11). A more active mode of life in predatory whales seems to make necessary the presence of a skin layer exposed to a considerable mechanical loading, when pursuing the prey. *D. delphis* and *L. obliquidens*, due to their comparatively small body-size, require a better thermo-isolation than large whales. Therefore, their subcutaneous fat tissue is relatively more developed than in other Odontoceti. *E. glacialis* has a well-developed subpapillary dermal layer with a non-adipose part in it.

The correlation between the dermis and the subcutaneous fat tissue must change according to the seasons of the year, and it is quite possible that in the dermis of all Odontoceti large accumulations of fat cells are formed in winter. We had no samples of the skin from whales obtained late in autumn or in winter, so our description refers to the summer period only.

Characteristic feeding habits of Balænopteridæ are connected with a considerable mobility of their skin, particularly on the ventral side of the body. Owing to this characteristic the net of elastic fibres is strongly developed in their skin. Besides, there are numerous specific bundles of elastic fibres (Fig. *A*17) scattered in the subcutaneous fat tissue. Moreover, in their subcutaneous muscles bundles of striated muscle fibres (Fig. *A*18) are surrounded by numerous elastic fibres (Fig. *A*19) running along them. In Odontoceti the net of elastic fibres in the skin is much less developed, bundles of the same fibres are absent in their subcutaneous fat tissue and there are no elastic fibres in subcutaneous muscles (Fig. *B*18). Active *D. delphis* and *L. obliquidens* possess dense nets of elastic fibres, and at the limit with their subcutaneous muscles plexuses of elastic fibres or even small bundles are to be found. In *E. glacialis* there is an ordinary net of elastic fibres, bundles of the latter being few in the subcutaneous fat tissue.



A scheme of the skin structure in Mystacoceti (*A*) and Odontoceti (*B*)
1, Epidermis; 2, dermis; 3, subcutaneous fat tissue. Epidermal layers: 4, corneous; 5, stratum spinosum or prickle cell layer; 6, basal; 7, dermal papillæ; 8, longitudinal septa of epidermal alveoli; 9, transversal septa of epidermal alveoli; 10, subpapillary dermal layer; 11, non-adipose part of subpapillary dermal layer; 12, blood vessels in dermal papillæ; 13, bundles of collagenic fibres; 14, fat cells; 15, epidermal projections extending into the tissue of dermal papillæ; 16, oval form of the diameter of epidermal alveoli; 17, bundles of elastic fibres; 18, bundles of striated muscle fibres in subcutaneous muscles; 19, elastic fibres surrounding bundles of striated muscle fibres in subcutaneous muscles

In subcutaneous muscles of the above-named whale elastic fibres are rare.

In Mystacoceti the skin of the snout has separate profusely innervated sensory hairs. In most Odontoceti there are no hairs in the post-embryonic period, but they are present in embryos. *Monodon* and *Delphinapterus* have no hairs at all even in the embryonic condition.

Besides all the characters indicated above the skin of Balænopteridæ is distinguished by the presence of longitudinal folds on the greater part of the body's ventral side, with the thickest epidermis, its corneous layer, the subpapillary dermal layer and the highest dermal papillæ at the tops of the folds. A gradual decrease in the size of those elements occurs towards the bottom of furrows between the folds.

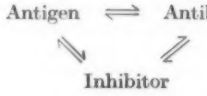
[1] Fjelstrup, A., *Zoologischer Anzeiger* (1888). Japha, A., *ibid.*, **29**, No. 14 (1906); *Zoologische Jahrbücher*, **24** (1907); *ibid.* (1910). Kükenthal, V., "Vergleichend-Anatomische und Entwickelungsgeschichtliche Untersuchungen an Waltieren" (Jena, 1889). Sokolov, W., *Bull. Soc. Naturalistes de Moscou*, Section Biologique, **60**, No. 6 (1955); Proc. Fifteenth International Congress of Zoology, London, 277 (1959). Weber, M., "Studien über Säugetiere. Ein Beitrag zur Frage nach dem Ursprung der Cetaceen" (Jena, 1886).

AN ANTIBODY INHIBITOR

By Dr. JACK G. MAKARI
Director of Research, Muhlenberg Hospital, Plainfield, New Jersey

IN our outlook on immunology in relation to disease we have specifically concentrated on antigens and antibodies, and we have attempted to explain all humoral host responses in these terms. Clearly there are areas which are not explained by this limited concept. What happens to the host before an antibody is produced? Does the body lack an early mechanism to tie up an invader before the antibody is formed? What does the body do in order to rehabilitate its reactive globulins or to stop the production of antibodies when the antigenic stimulus disappears? Furthermore, we have mounting evidence to show that auto-antibodies directed against the host's own constituents are being formed in even so-called normal humans. How is this auto-antibody handled? How is it tied up so that it cannot reach its target? Furthermore, how do we explain psychosomatic influences, hormonal factors, and the impact of stress on the organism in terms of immunology?

All these factors require that we expand our concept of host defences to include a triad of antigen, antibody, and inhibitor as follows:



I have for many years been interested in this mechanism. I have described an inhibitor earlier[1], studied some of its behaviour *in vitro* as demonstrated by its capacity to inhibit flocculation of normal globulins in the presence of cephalin cholesterol emulsion, and investigated its behaviour by means of serial cephalin flocculation curves[2].

Previously I found that immunization resulted in an increase in this inhibiting factor prior to and independent of the specific antibody response. Drugs (such as antimalarials), alcohol, emotional excitement, stress and even physiological mechanisms, such as food and physical exercise[3], all influenced this response. Furthermore, in the study of host-parasite interaction, as occurs in malarial infection, it was apparent that this inhibitor was a resistance factor of vital significance to the host[3].

In this present study evidence is presented to show the dual function of this inhibitor. For this same inhibitor, which can inhibit the flocculation of globulins in the presence of cephalin–cholesterol–ether emulsions, is, in fact, an antibody inhibitor, capable of preventing the fixation of antibodies to uterine segments in the Schultz–Dale test.

In the preliminary experiment shown in Table 1, the source of antibodies for this *in vitro* fixation to uterine segments was the serum from patient *P.*, a case of active melanoma tumour with metastasis, and from patient *B.*, a case of leukoplakia without any evidence of tumour. The two uterine horns (obtainable from a healthy, unsensitized guinea pig) were then removed. Each of these horns was then cut in half into two segments. The resulting four segments were then ready for coating. Two of these uterine horns were immersed in 0·5 ml. of serum diluted with 0·5 ml. of Tyrode solution; the other two segments were immersed in 0·5 ml. of serum diluted with 0·5 ml. of inhibiting factor. To each of these solutions (to be used for coating) 1·5 ml. Tyrode solution was added. Contact of the serum–Tyrode mixture with uterine segments was done at 0–5° C. (that is, surrounded by crushed ice) for 1 hr. with air bubbled through the mixture throughout the period of contact.

The coated uterine segments were then used in a Schultz–Dale bath[4–6]. The antigens used to release

Table 1. Influence of Normal Inhibitor on Fixation to Uterine Segments (as shown by the Schultz–Dale Test) of Antibodies from Sera of Two Patients: One with Tumour and the Other in the Pre-tumour Stage
Contraction index$_{1,000}$ values are given below

Releasing antigen		Source of serum antibody Pt. *B.*—Leukoplakia				Source of serum antibody Pt. *P.*—Melanoma			
Type	Amount in ml.	Tyrode		Inhibitor		Tyrode		Inhibitor	
		Horn I	Horn II	Horn III	Horn IV	Horn I	Horn II	Horn III	Horn IV
Normal serum	0·5 ml.	0	0	0	0	0	0	0	0
Normal antigen	0·4 ml.	0	0	0	0	0	0	0	0
Carcinoma antigen (supernatant)	0·4 ml.	1,000	1,000	180	0	1,000	830	1,000	1,000
Carcinoma antigen (supernatant)	0·4 ml.	0	260	0	0	0	0	450	590
Melanoma antigen (supernatant)	0·4 ml.	1,000	1,000	0	0	920	920	1,000	1,000
Melanoma antigen (supernatant)	0·4 ml.	1,000	1,000			0	0	1,000	890

Table 2. Effect of Normal Antibody Inhibitor on Fractions from Pooled Sera containing Antibodies to Tissue Antigens, Amino-acids and Other Key Substances
Contraction index values are multiplied by 1,000

	Antibodies to tissue and serum antigens				Antibodies to amino-acids				Antibodies to other key substances			
	No. of tests	*A* Mean C.I. with Tyrode	*B* Mean C.I. with normal inhibitor	*B/A* Ratio of inhibition	No. of tests	*A* Mean C.I. with Tyrode	*B* Mean C.I. with normal inhibitor	*B/A* Ratio of inhibition	No. of tests	*A* Mean C.I. with Tyrode	*B* Mean C.I. with normal inhibitor	*B/A* Ratio of inhibition
Benign 50 per cent fraction	19	102	93	0·91	7	0	0		17	110	52	0·47
Ca. local 50 per cent fraction	22	188	94	0·50	16	290	96	0·33	51	111	36	0·32
Ca. local extension 50 per cent fraction	27	161	50	0·31								
Ca. Metastatic (+ test) 50 per cent	25	162	98	0·60	6	0	0					
Ca. Metastatic (− test) 50 per cent	29	127	54	0·43								
Ca. Cure 50 per cent	20	97	26	0·27								
Ca. local 33 per cent	11	89	154	1·73								
Ca. local extension 33 per cent	13	119	170	1·43								
Ca. Metastatic (+ test) 33 per cent	19	221	105	0·48								
Ca. Local 66 per cent	11	199	35	0·18	7	0	0		12	45	35	0·78
Ca. Local extension 66 per cent	10	18	92	5·11								
Ca. Metastatic (+ test) 66 per cent	24	210	177	0·84								
Ca. Metastatic (− test) 66 per cent	24	431	301	0·70	11	314	224	0·71	15	124	108	0·87

the reactions were supernatant solutions obtained from 20 per cent suspensions of carcinoma and melanoma tissues in isotonic sodium chloride, after removal of the sediments obtained at 14,000 r.p.m. The Tyrode solution used has half-strength calcium and ten times the normal amount of magnesium (sodium chloride, 9·0 gm.; potassium chloride, 0·20 gm.; calcium chloride, 0·05 gm.; magnesium chloride, 0·10 gm. (of crystalline magnesium chloride use 0·21); glucose, 1·00 gm.; sodium bicarbonate 1·00 gm.; sodium dihydrogen phosphate, 0·05 gm.; demineralized distilled water, 1 l.).

A corresponding normal antigen also used to release the reactions was prepared in the same way from several normal tissues (œsophagus, stomach, colon, lung). These normal tissues, which were obtained from a young victim of a car accident at autopsy, were pooled.

The results of this experiment (shown in Table 1) indicate the presence of two types of antibodies to tumour antigens: (1) those antibodies inhibited by normal inhibitor and present in the serum from the leukoplakia case: (2) those antibodies not inhibited by normal inhibitor found in the serum of the patient with active melanoma.

The finding that specific desensitization is possible to carcinoma antigens indicates the antigen–antibody basis of this reaction.

In the other experiments shown in Table 2 sera from 26 individuals with benign and malignant tumours were pooled according to the nature of the tumour, the state of tumour spread and the result of my tumour skin test[7,8] as follows: (1) Benign tumours—tumour skin test +, mean age 39·8 (4 individuals). (2) Carcinoma—early localized—tumour skin test +, mean age 60·3 (3 individuals). (3) Carcinoma—stage of local extension—tumour skin test +, mean age 47·5 (6 individuals). (4) Carcinoma—stage of metastasis—tumour skin test + mean age 54·2 (5 individuals). (5) Carcinoma—stage of metastasis—tumour skin test −, mean age 63 (4 individuals). (6) Carcinoma—cure (survival beyond 5 years)—tumour skin test −, mean age 69 (4 individuals).

These various pooled sera were then fractionated with ammonium sulphate into the following fractions: 33, 50 and 66 per cent. The precipitate in each case was dissolved in isotonic sodium chloride and brought to the original volume of the serum. Each serum fraction was then dialysed against running tap water, distilled water, sodium chloride 1 per cent, and phosphate buffer (sodium chloride, 6·8 gm.; sodium hydrogen phosphate, 0·81 gm.; potassium dihydrogen phosphate, 1·04 gm.; distilled water, 1,000 ml.: *p*H 6·8). The various fractions were then used for *in vitro* coating of uterine segments in the presence or absence of inhibiting factor from a normal blood donor as in the previous experiment.

The inhibiting factor was isolated by the following procedure: to the supernatant after the extraction of 66 per cent fraction, 2*N* sulphuric acid in 50 per cent ammonium sulphate was added slowly with mechanical stirring until the *p*H became 4·8. The precipitate was separated by centrifugation for 45 min. at 10,000 r.p.m. and discarded. The supernatant was then saturated with crystals of ammonium sulphate with continuous mechanical stirring for 1–2 hr. This was left over-night and then centrifuged for 45 min. at 10,000 r.p.m. The supernatant was discarded. The precipitate was dissolved in isotonic sodium chloride solution enough to bring it back to the original volume of serum. It was then dialysed in the same way as the other blood fractions from the diseased sera.

The coated uterine segments were then used in the Schultz–Dale test. To trigger the anaphylactic reactions, tissue and serum antigens obtained from normal and tumorous subjects were used, as well as various amino-acids and other key substances (such as deoxyribonucleic acid, adenylic acid, thymine, purine, pyrimidine, etc.).

Earlier work[9] has demonstrated the presence of antibodies to amino-acids (*a–a* antibodies) and antibodies to other key substances (*k–s* antibodies) in the

sera of patients with cancer. An attempt was made in these experiments to see whether the inhibiting factor is effective in inhibiting *a–a* and *k–s* antibodies as well as antibodies to tissue and serum antigens.

The resulting specific anaphylactic reactions were then compared to the contraction of the same uterine segment when 10γ of histamine were added to the bath. This ratio of the specific contraction to that of histamine, that is, the contraction index, was further multiplied by 1,000 to obtain contraction index 1,000.

A ratio of inhibition was obtained by dividing the mean contraction index in the presence of normal inhibitor by the mean contraction index in the presence of Tyrode solution. The effect of inhibiting factor from a normal subject on the fixation to the uterine segments of antibodies, found in the various fractions from pooled sera of patients with tumours, is shown in Table 2. It is apparent from this Table that most of the antibody globulins studied are amenable to the inhibiting action of inhibiting factor (that is, preventing the antibody fixation to the uterine segment) as evidenced by inhibition ratios less than 1·0. Other antibody globulins, however, are not inhibited by inhibiting factor. Instead, a reversal seems to take place so that these antibodies are more fixed to the uterine segments in the presence of inhibiting factor. Thus all the globulins in the 50 per cent fractions could be inhibited as indicated by inhibition ratios less than 1·0 (the lowest value being that of a carcinoma cure pool).

Of the antibody globulins precipitated at 33 per cent only one out of the three fractions tested are inhibited by inhibiting factor (namely, that of carcinoma metastatic tumour skin test +). The other two 33 per cent fractions from carcinoma local and carcinoma local extension show a reversal of inhibition in the presence of inhibiting factor indicating an abnormality of these latter two antibodies.

Of the antibody globulins precipitated at 66 per cent three out of the four showed inhibition by inhibiting factor (namely, those from fractions studied from carcinoma—local, carcinoma—metastatic tumour skin test +, carcinoma—metastatic tumour skin test −). The fourth serum fraction, representing antibody globulins precipitated at 66 per cent and obtained from the carcinoma—local extension pool, showed a marked reversal of inhibition to inhibiting factor (inhibition ratio being 5·11).

All the *a–a* and *k–s* antibodies studied seem to be amenable to inhibition by inhibiting factor. Again, those associated with the 50 per cent fraction seem to be more amenable to inhibition by inhibiting factor than those associated with the 66 per cent fraction.

These results demonstrate the presence of an antibody inhibitor in normal serum capable of inhibiting the fixation of antibody globulins to smooth muscle in the Schultz–Dale test. This same inhibitor is also capable of inhibiting the flocculation of normal globulins in the presence of cephalin–cholesterol–ether emulsions.

In some stages of cancer, inhibiting factor is capable of inhibiting antibodies associated with certain serum fractions, while in other stages, inhibiting factor is incapable of inhibiting antibodies associated with certain serum fractions. In these latter cases there seems to be a reversal of function so that even a normal inhibiting factor will result in increased, rather than decreased, fixation of antibodies to smooth muscle.

Other than this immunological defect in antibodies, found in certain serum fractions in cancer, there seems to be a defect in the inhibiting factor itself found in cancer sera. Evidence for this finding will be presented elsewhere.

I believe that this new concept of antibody inhibitor will prove to be not only of academic interest but also of assistance in allergic diseases, autoimmune disease and in the study of the ageing process and cancer.

[1] Makari, J. G., *Nature*, **160**, 201 (1947).
[2] Makari, J. G., *J. Trop. Med. and Hyg.*, **49**, 70 (1946).
[3] Makari, J. G. (unpublished results).
[4] Makari, J. G., *Brit. Med. J.*, **2**, 1291 (1955).
[5] Makari, J. G., *Brit. Med. J.*, **2**, 353 (1958).
[6] Makari, J. G., *J. Amer. Geriatrics Soc.*, **7**, 611 (1959).
[7] Makari, J. G., *Amer. J. Pub. Health*, **49**, 687 (1959).
[8] Makari, J. G., *J. Amer., Geriatrics Soc.*, **8**, 4, 16 (1960).
[9] Makari, J. G., *J. Geront.*, **14**, 494 (1959).

A BIVALVE GASTROPOD

By Dr. L. R. COX, O.B.E., F.R.S., and Dr. W. J. REES
British Museum (Natural History), London, S.W.7

THE recent discovery of a remarkable mollusc, described as *Tamanovalva limax* gen. et sp. nov. by S. Kawaguti and K. Baba[1], calls for special notice, as this organism, while undoubtedly a gastropod, possesses a bivalve shell; it is therefore of considerable interest to malacologists who are concerned with the possible origins of bivalve molluscs.

This unique type of gastropod, of which 200 or more specimens have been found, occurs in the sublittoral zone at Bisan Seto on the Inland Sea of Japan. The largest shells are 7 mm. long. The main features of the animal are :

(1) A slug-shaped head–foot mass which can be protruded from the shell and extends well to the front of it when the animal is crawling; it can be completely withdrawn between the two valves, which close as in a lamellibranch. The foot is narrow and has a flat sole which can be folded longitudinally along the median line.

(2) Two stout rhinophores and two small lobiform oral tentacles on the well-developed head, and eyes which lie close together on a small median projection some way from its extremity.

(3) A radula of the sacoglossan type, in which only one tooth is in operation at a time, when the animal is feeding on its host plant (the alga *Caulerpa*).

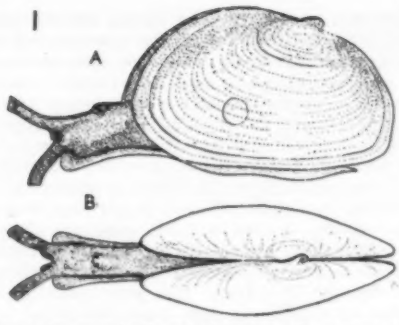
(4) A male genital aperture with penis, located far forward on the right side of the body, just behind the head.

(5) A bilobed mantle open along the ventral margins.

(6) A single gill, formed of a series of thin lamellæ as in the sacoglossan genera *Oxynoe* and *Lobiger*, and situated under the right mantle lobe.

(7) The anus opening into the mantle cavity well to the rear.

These structures and others that need not be mentioned are indicated in Figs. 1 and 2. There are no



Figs. 1*a*, *b*. *Tamanovalva limax*: *a*, lateral view from left; *b*, dorsal view: note the rhinophores, eyes and protoconch (× *c*. 6·6). (After Kawaguti and Baba)

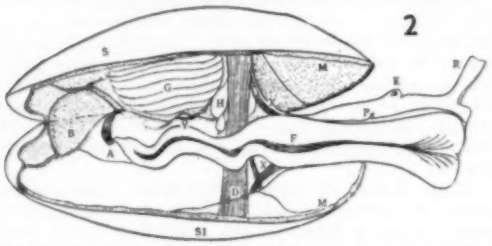


Fig. 2. *Tamanovalva limax*: ventral view (× 8). *A*, anus; *B*, hypobranchial gland; *D*, adductor muscle; *E*, eye; *F*, foot; *G*, gill; *H*, heart; *M*, mantle edge; *P*, penis pore; *R*, rhinophore; *S*, right valve of shell; *Sl*, left valve of shell; *V*, vaginal pore; *X*, head retractor muscle. (After Kawaguti and Baba)

siphons and no parapodia. The nervous system is not described.

The most remarkable feature is the shell, consisting of two valves which are equal in size and shape except for a small helicoid spiral structure at the apex of the left valve. There is a single well-developed adductor muscle which connects the two valves of the shell slightly in front of the middle of its length. The valves are joined along the hinge-line by an 'invisible' (? transparent) ligament; but there are no hinge-teeth.

Eggs are laid in sausage-shaped masses about 10 mm. long. The developing embryo passes through trochophore and veliger stages, and when the veliger hatches out it has a bilobed velum, a helicoid shell and an operculum. It is the larval shell that is preserved, as a protoconch, at th eapex of the left valve of the adult shell; it appears to be sinistrally coiled although the animal is dextrally organized (a feature of the typical opisthobranch protoconch).

The authors conclude that *Tamanovalva* is an opisthobranch gastropod belonging to the order Sacoglossa, as shown particularly by its slug-like body with two stout rhinophores and the nature of its radula. In view of its remarkable bivalve shell they have placed it in a new suborder Tamanovalvacea.

That this animal is a gastropod is indisputable, and its reference to the order Sacoglossa seems well justified. The opisthobranchs are now considered to be the most advanced sub-class of the Gastropoda, and many have contrived to counteract the disadvantages of torsion and asymmetry possessed by their prosobranch ancestors by the acquisition of secondary symmetry, of which *Tamanovalva* surely provides a further and most striking instance. It is to be interpreted as an evolutionary experiment in a subclass not represented among the earlier known gastropods and in an order of which no fossil representatives have yet been recognized. The mere fact, however, that it demonstrates the possibility of a bivalve and almost symmetrical shell becoming developed in a normally univalve group lends support to the theory that the Bivalvia (Lamellibranchia) were derived originally from primitive univalve molluscs. Whereas, however, it has been supposed that this event involved the bending and hinging of a symmetrical, cap-like shell along its median line and the fusion of pallial muscles to form the two adductor muscles[2], the origin of the second valve and of the adductor muscle of *Tamanovalva* cannot be satisfactorily discussed until the post-embryonic ontogeny of this form has been investigated. It would appear that the protoconch-bearing left valve represents the original univalve shell and that the second valve is an extension which presumably arises by bilobation of the mantle at a relatively early stage in ontogeny (probably at the time of metamorphosis) and becomes hinged. Being quite independent of the foot, it clearly cannot arise from the operculum, which presumably, as in most other opisthobranchs, atrophies at an early stage in development.

Kawaguti and Baba refer briefly to *Edenttellina* Gatliff and Gabriel[3], a supposed lamellibranch which, like their new genus, is characterized by the presence of a small coiled larval shell at the tip of one valve; but they consider it to be quite distinct. We are by no means convinced about this. The type-species of *Edenttellina*, *E. typica*, was first discovered along the coast of Victoria and has since been found off South Australia and Western Australia. A second species, *E. corallensis*, dredged off Queensland within the Great Barrier Reef, has been described by Hedley[4], who at the same time has also re-described the type species. It seems significant that, according to that author, *E. typica* "lives in shallow water a little below the level of low tide" and that he had "commented on the absence from *Edenttellina* of characteristic pelecypod features and suggested that possibly it might be the internal shell of a tectibranch" (tectibranchs are opisthobranch gastropods).

Hedley's figures of *E. typica*, which are more satisfactory than the rather poor photographs that illustrated the original description of the species, represent a small shell closely resembling *Tamanovalva* in shape. Although identified as a right valve from its outline (on the assumption that it belonged to a lamellibranch), the valve bearing the coiled larval shell at its tip is very similar to the corresponding valve (known to be the left one) of the Japanese form. In the original description of *E. typica* the shell was described as being without hinge-teeth, but afterwards, when better specimens were obtained, a small tooth-like structure was detected in both valves. Similar but possibly more obscure structures may have been overlooked in the Japanese species. The position of the adductor scar or scars has not been observed in *Edenttellina*, while Hedley's statement that the soft parts of the animal are unknown appears still to hold good. It is, therefore, now all the more desirable that a special search for living specimens should be made in Australian waters, to ascertain whether or not this is a gastropod genus synonymous with *Tamanovalva*.

There is a further point, of special interest to palæontologists. As pointed out in Hedley's paper, a rare species found fossil in the Eocene of the Paris Basin appears to be a representative of *Edenttellina*. It was known to Deshayes, who had assigned the

MS. name *Ludovicia squamula* gen. et sp. nov. to it, but was first described by Cossmann in 1888[5], by which time the name *Ludovicia* had already been published in connexion with an entirely distinct organism. The Paris Basin fossil is a small, thin-shelled bivalve of the same general trapeziform shape as *Edenttellina* and *Tamanovalva*, and young specimens have, at the tip of the left valve, a small spiral larval shell which is later lost. Although he included it in the lamellibranch family Galeommidae, Cossmann remarked, "j'aurais pu croire qu'il s'agissait d'un gastropode". If it is accepted as an *Edenttellina*, and if the latter proves to be identical with the newly described Japanese genus, we shall know for the first time that the opisthobranch order Sacoglossa existed so long ago as the Eocene.

The authors' full report on the remarkable aberrant form described in their paper will be eagerly awaited.

[1] *Biol. J. Okayama Univ.*, **5**, 177 (1959).
[2] Yonge, C. M., *Trans. Roy. Soc. Edin.*, **62**, 444 (1953).
[3] *Proc. Roy. Soc. Victoria*, N.S. **24**, 190, pl. 46, Figs. 5, 6 (1911).
[4] *Proc. Malac. Soc. Lond.*, **14**, 76 (1920).
[5] *Ann. Soc. Roy. Malac. Belg.*, **22**, 45, pl. 2, Figs. 21, 22.

THE SECONDARY PAIRING OF BIVALENTS WITH GENETICALLY SIMILAR CHROMOSOMES

By Dr. RALPH RILEY
Plant Breeding Institute, Cambridge

IN some allopolyploid plants, at first metaphase of meiosis, the bivalents are not randomly distributed but occur together in pairs or groups. This secondary pairing has been assumed to take place between bivalents composed of genetically similar pairs of chromosomes[1,2], but this assumption has apparently never been tested. Material suitable for such a test can, however, now be produced in the allohexaploid common wheat (*Triticum aestivum* $2n = 6x = 42$), in which secondary pairing may be occasionally observed.

The material used in the present investigation was developed from three parental lines in the variety Chinese Spring, generously supplied by Dr. E. R. Sears. These had chromosomes III, XII and XVI, in turn, represented by a pair of telocentric chromosomes for one arm, the other arm being completely deficient. Chromosomes III, XII and XVI are homœologous[3], that is, genetically equivalent chromosomes, belonging respectively to the *B*, *A* and *D* genomes derived from the original diploid ancestors of wheat[4].

The three lines were crossed together in pairs to produce *F*1's with one normal and one telocentric chromosome for each of two pairs. Thus, in the cross between the line with chromosome III telocentric and the line with chromosome XII telocentric, the hybrid had one telocentric member of the III and of the XII pair, and one normal member of each pair.

Meiotic pairing between the normal and telocentric chromosomes led to the formation of heteromorphic, rod-shaped bivalents, since chiasma-formation could take place only in one arm. The heteromorphics were readily recognizable in meiotic preparations, so that the relative positions of the two bivalents at first metaphase could be recorded.

The preparations analysed were Feulgen-stained squashes of pollen mother cells from anthers fixed acetic alcohol. Only cells having strictly linear arrangements of chromosomes were scored (Fig. 1), and the scoring recorded the number of bivalents which intervened between the two recognizably heteromorphic pairs. This method is artificial in that a structure which was originally two-dimensional, the metaphase plate, is represented as one-dimensional. However, it seems unlikely that squashing resulted in systematic distortions of the relative spacings of bivalents on the original plates. Moreover it should be indicated that the score of the number of bivalents intervening between the heteromorphic pairs is merely a measure of the degree of separation, and does not represent the number of bivalents by which they were separated on the original plate. With these reservations, however, the technique of recording seems satisfactory.

The data derived from scores of the three types of *F*1's with two heteromorphic bivalents are recorded in Table 1. There are no significant differences between the three distributions (III/XVI – III/XII, $\chi^2_{(14)} = 24{\cdot}56$, $P = 0{\cdot}1$–$0{\cdot}5$; III/XVI – XII/XVI, $\chi^2_{(15)} = 19{\cdot}03$, $P = 0{\cdot}3$–$0{\cdot}2$; and XII/XVI – III/XII, $\chi^2_{(14)} = 18{\cdot}86$, $P = 0{\cdot}2$–$0{\cdot}1$), so the data have been summed. The summed distribution falls off steeply from a clear maximum of cells with the heteromorphic bivalents adjacent, to minimum frequencies with the heteromorphics at opposite ends on the plate. The observed distribution has been tested against that expected from the random alignment of bivalents. This may be calculated from the generalized formula $\frac{2(n-1-r)}{n(n-1)}$, where n is the total number of bivalents and r is the number of intervening bivalents, for which I am indebted to Dr. W. F. Bodmer. The random distribution is a straight line which in the present instance runs from a frequency of 0·0952

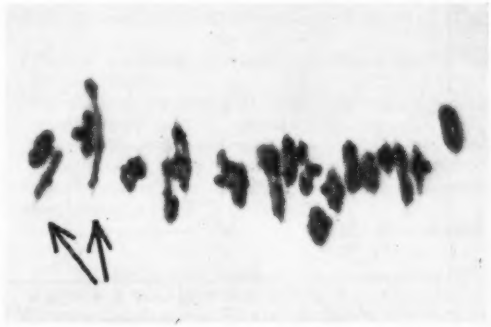


Fig. 1. First metaphase of meiosis in a plant of *T. aestivum* with one telocentric member of both the chromosome III and XVI bivalents. There is one intervening bivalent between the heteromorphic bivalents III and XVI, which are arrowed. (× 1,250)

Table 1. Number of Bivalents separating Homœologous Pairs in First Metaphase Cells

No. intervening bivalents	Cells per intervening class					χ^2
	III+XII marked	III+XVI marked	XII+XVI marked	Total	Random expectation	
0	21	40	23	84	39·22	62·55
1	9	21	15	45	37·29	1·59
2	8	15	12	35	25·30	1·12
3	9	10	8	27	33·33	1·20
4	6	10	6	22	31·39	2·81
5	6	11	8	25	29·42	0·66
6	6	9	6	21	27·48	1·52
7	6	8	5	19	25·50	1·66
8	9	3	5	18	23·57	1·32
9	9	11	5	25	21·59	0·54
10	5	4	1	10	19·61	4·71
11	6	4	5	15	17·67	0·40
12	4	3	4	11	15·69	1·40
13	5	3	3	11	13·72	0·54
14	3	6	1	10	11·78	0·27
15	4	4	3	11	9·81	0·14
16	3	3	6	12	7·87	2·17
17	1	2	2	5	5·89	
18	3	4	1	8	3·96	4·35
19	0	3	3	6	1·98	
				19*	11·83*	
Total	123	174	122	412	412·00	88·95

$\Sigma\chi^2_{(17)} = 88{\cdot}95$; $P = < 0{\cdot}001$

* Classes summed to increase the value of the expected.

with no interveners to 0·0048 with 19 interveners (Fig. 2).

There is a marked and highly significant deviation of the observed distribution from random ($\chi^2_{(17)} = 88{\cdot}95$, $P = < 0{\cdot}001$), and the largest single deviation is in the class with no intervening bivalent. Indeed, when the ratio of observations of the combined classes 1 to 19 : 0 is tested against random, the deviation is highly significant ($\chi^2_{(1)} = 56{\cdot}51$, $P = <0{\cdot}001$). Thus the major disturbance of the random distribution results from the heteromorphic bivalents occurring together too frequently.

If this represents secondary pairing, in the present experimental situation the apparent attraction between the marked homœologues may well have been affected by the deficiency of an arm from the telocentric member of each pair. If genetically corresponding arms were missing from the marked pairs, the amount of structurally similar material would be reduced, depending on the relative lengths of the arms of each chromosome, by about a quarter. Similarly, if the arms missing in each pair were not genetically equivalent, there would be only half the normal amount of structurally similar material. Consequently, if pairing depends on the degree of structural similarity, results based on pairs with telocentric members may under-estimate normal attraction.

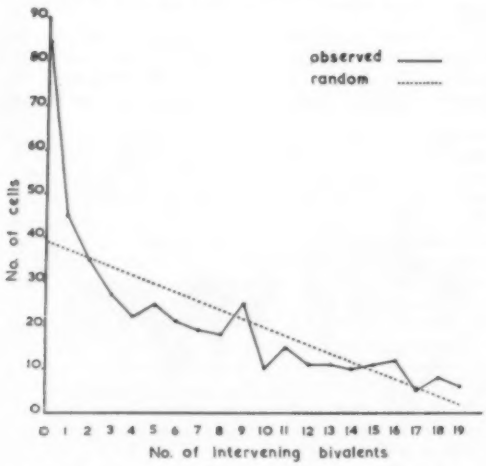


Fig. 2. Observed and random distributions of cells with the heteromorphic homœologous bivalents separated by 0–19 unmarked bivalents

Also the degree of secondary pairing might have been diminished because the marked bivalents were always rods, and rod bivalents probably move less freely than rings during the metaphase congression. Moreover, wheat is a hexaploid in which secondary pairing is ideally possible between all three bivalents involving chromosomes III, XII and XVI. However, in the material so far available, only two of these pairs have been simultaneously recognizable; consequently the influence of the unmarked pair could not be determined. The excess of the class with one bivalent intervening may, however, be due to the separation of the marked pairs by their unmarked homœologue.

The excess of the combined classes with 17, 18 and 19 interveners may reflect a slightly non-random component in the arrangement of rod bivalents. Possibly they were more often at the periphery of the original plate, or their positions may have influenced the orientation of the plate on squashing. However, the majority of adjacent heteromorphic bivalents were in the interior rather than at the ends of the plates, so that this factor has probably not greatly influenced the recorded frequency of adjacent pairs. In subsequent work with marked non-homœologous as well as homœologous pairs, and in material with all three homœologues marked, this problem may be clarified.

Nevertheless, despite the intrusion of other disturbance, the considerable excess of immediately adjacent homœologous pairs seems to be reasonable evidence that secondary pairing brings genetically similar bivalents together, as had been supposed. If this is so, homœologous pairs must be attracted together following the wide dispersal of bivalents throughout the nucleus during diakinesis. However, the chances of secondary association very likely depend upon the relative proximities of homœologues during diakinesis. In wheat there are probably zygotene attractions between all six homœologous chromosomes but, in the presence of chromosome V, there is complete synapsis only between homologues[5]. If as a consequence of zygotene attractions homœologous bivalents are neighbours in early prophase, they are not likely to be so widely separated at diakinesis as non-neighbours, so that secondary pairing at metaphase will readily follow.

Evidence of secondary pairing between bivalents III, XII and XVI is a useful supplement to the demonstration of similarity in their genetic activity[3]. It is a further indication of the evolutionary proximity of these three chromosomes, and indeed of the genomes of which they are part. The extent of genetical and structural triplication of chromosomal material of wheat is thus re-emphasized in a way which re-asserts the significance of the genetic regulation causing wheat to form only bivalents, and no multivalents, at meiosis.

[1] Darlington, C. D., *J. Genet.*, **19**, 215 (1928).
[2] Lawrence, W. J. C., *Cytologia*, **2**, 352 (1931).
[3] Sears, E. R., *Res. Bull. Mo. Agric. Exp. Sta.*, 572 (1954).
[4] Okamoto, M., *Wheat Inf. Service*, **3**, 6 (1957).
[5] Riley, R., and Chapman, V., *Nature*, **182**, 713 (1958).

LETTERS TO THE EDITORS

PHYSICS

Surface-controlled Bulk Conductivity in Organic Crystals

In an earlier paper[1] experiments were described which demonstrated hole injection from unexcited and optically excited electrode layers into an adjacent anthracene crystal, resulting in a large increase in the current passing through the bulk of the crystal. In the present communication, the interaction between an optically excited anthracene crystal and its adjacent electrode is reported.

The experimental techniques used have been described in detail elsewhere[2,3]. The general procedure was to use an electrolyte as the electrodes for thin (10μ) single crystals of anthracene. The incident light passed through the electrolyte before striking the crystal face. The current through the crystal was measured with 1M sodium iodide and 1M sodium chloride solutions in contact with the illuminated side of the crystal, using 3650 Å. light which was transmitted by the electrolyte but completely absorbed by the anthracene.

at least part of the current measured with the illuminated side negative is due to an interaction at the unilluminated electrode which produces positive holes at that face. The total $i-$ current would, therefore, be made up of the flow of these holes from the face not directly illuminated as well as electrons from the illuminated face. On this basis, the flow of electrons in $i-$ in the case shown in column 6 must be less than 0·3 per cent of $i+$.

In all cases, the photocurrents were almost saturated at 50 V. or lower. The dark current did not exhibit saturation.

The quantum efficiency for the photocurrent when the illuminated face of the crystal was in contact with sodium chloride solution was one carrier liberated into the anthracene crystal for thirty quanta falling on the face of the anthracene. In the case where the illuminated face was in contact with sodium iodide solution, the quantum efficiency was one carrier liberated into the anthracene for 390 quanta falling on the face of the anthracene.

In general, these results may be understood by assuming that the observed photocurrents are a

Table 1. Photoconductivity in Anthracene using Sodium Chloride and Sodium Iodide Electrolyte Electrodes

Applied voltage	(1) Side A : NaI B : NaI Dark current	(2) A : NaI B : NaCl Dark current		(3) A : NaI B : NaI Photocurrent		(4) A : NaI B : NaCl Photocurrent		(5) B : NaI A : NaI Photocurrent		(6) B : NaCl A : NaI Photocurrent	
	$i+ = i-$*	$i+$	$i-$	$i+$	$i-$	$i+$	$i-$	$i+$	$i-$	$i+$	$i-$
(Volts)	× 10^{12} amp.	× 10^{12} amp.		× 10^{9}† amp.		× 10^{9}† amp.		× 10^{9}† amp.		× 10^{9}† amp.	
2				10	0·33	6·5	2·1	4·1	0·40	13·6	0·25
4	0·3	0·2		21	0·40	14·5	3·5	11·5	0·52	41·6	0·40
8	0·5	0·5		29	0·46	22·5	4·3	17·5	0·63	148	0·60
15	0·9	1·1		36	0·52	29	4·5	21·5	0·72	240	0·80
30	1·7	2·5	3·5	40	0·58	33	4·7	24·0	0·81	312	1·05
50	3·0	4·4	6·0	42	0·62	35	5·0	25·2	0·87	328	1·15

Area of crystal, 0·1 cm.². Intensity of 3650 Å. light, 320μ watt/cm.². All solutions are 1 M in concentration.
* $i+$ and $i-$ refer to currents measured when the polarity of the side illuminated by light was positive and negative respectively.
† Note change in scale.
The side illuminated by the light is listed at the top of each column in the table.

The experimental results are given in Table 1. The most interesting result was that the magnitude of $i+$ when the illuminated side of the anthracene was in contact with sodium chloride solution (column 6) was 13 times that of $i+$ when this side was in contact with sodium iodide solution (column 5). The current $i-$ did not change much.

It was also noted that the ratio $i+/i-$ in the case shown in column 6 was 285 as compared with a ratio of 7 in the case shown in column 4. The large value of $i-$ in column 4 was felt to be due in most part to an effect of fluorescent light on the anthracene–sodium chloride electrode face not directly illuminated. Scattering effects were ruled out by shielding the unilluminated face. Using identical sodium iodide electrodes, the ratio $i+/i-$ at 50 V. was 68 (column 3). The latter experiment was repeated with no change other than shifting the illumination from one face of the crystal to the opposite face. The ratio $i+/i-$ was 29 (column 5), indicating some crystal asymmetry. The change from sodium chloride to sodium iodide in the electrolyte at the unilluminated side decreases the $i-$ current, however, by a factor of 8, whereas the $i+$ current is almost unchanged (compare columns 3 and 4).

This dependence of $i-$ on the nature of the electrolyte in contact with the crystal face indicates that result of the creation of an exciton by the light absorbed in the anthracene and its subsequent diffusion to the adjacent electrode, where it undergoes a dissociation into an electron and a positive hole. The latter then moves through the anthracene under the action of the electric field. Any event which will quench the exciton or inhibit its subsequent dissociation will diminish the photocurrent. Apparently such quenching occurs at the electrode by the I^- and its quenching action is greater than that of the Cl^-.

A more complete discussion of these results will be given shortly.

The work of Mr. Peter Gordon, who made most of these measurements, is greatly appreciated.

Grateful acknowledgment is made of the assistance of the Office of Naval Research (*NONR* 285(41)) in this work and of the Air Force Cambridge Research Center, Contract *AF*19(604)–3888.

H. Kallmann
M. Pope

Physics Department,
New York University,
Washington Square, New York.

[1] Kallmann, H., and Pope, M., *J. Chem. Phys.*, **32**, 300 (1960).
[2] Kallmann, H., and Pope, M., *Rev. Sci. Instr.*, **29**, 993 (1958).
[3] Kallmann, H., and Pope, M., *Rev. Sci. Instr.*, **30**, 44 (1956).

Emission Spectroscopy with Infra-Red Double-Beam Spectrophotometers

Some time ago, K. A. Fischer and G. Brandes[1] demonstrated that it is possible to obtain useful emission spectroscopic data with an infra-red double-beam spectrophotometer, if the sample placed in the beam path of the spectrophotometer is warmed up with a closed sample beam shutter. Instead of the quotient of the radiation intensity passed by the object and of the unattenuated radiation intensity, the instrument now scans the quotient of the inherent radiation intensity of the object and of the reference beam intensity.

The sensitivity of this method can be increased 100 times, if the intensity of the reference beam is attenuated by means of a Venetian shutter by the same factor. Thus it is possible to record also the relatively weak emission maxima of moderately heated gases.

Fig. 1 shows the emission spectrum of ammonia, heated up to 160° C., between 9 and 12μ, recorded with a ten-fold reference beam attenuation. The thickness of the ammonia sample was 5 cm. The spectrum was recorded with a Model 21 Infra-red Spectrophotometer of Bodenseewerk Perkin-Elmer and Co., GmbH. The attenuation of the reference beam was effected by a reference beam attenuator designed as an accessory to this instrument, the object being warmed up in a heatable gas cell, which likewise constitutes a usual spectrophotometer accessory though serving other purposes otherwise. The spectral dispersion was effected with a sodium chloride prism; the slit-width was controlled with the 'slit programme 800'. This slit programme provides a slit-width of 55μ at a wave-length of 10μ. Because of the low energy striking the thermocouple, the instrument was operated with maximum amplification, maximum time constant and a scanning speed of 5×10^{-4} μ/sec. The spectrum distinctly shows the emission maxima assigned to the absorption bands of the ammonia. Fig. 2 shows the absorption spectrum of the same sample, which was scanned with the same slit programme, however, without reference beam attenuation. It becomes obvious that the frequency conformity of the emission and absorption maxima has been realized quite well. The intensity ratios between the individual maxima are, however, considerably changed.

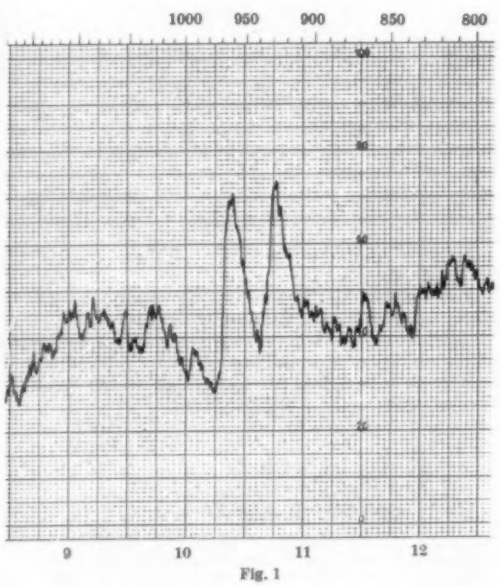


Fig. 1

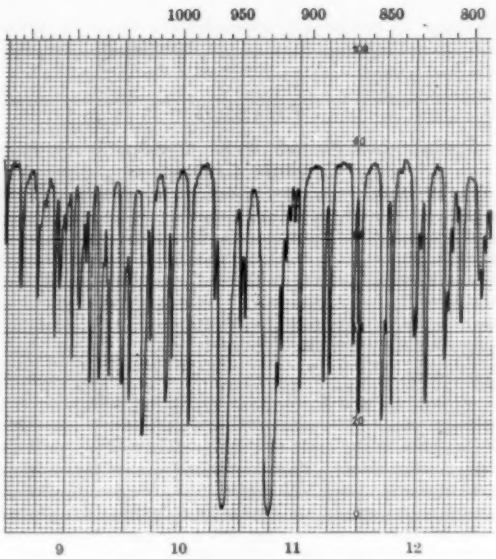


Fig. 2

J. Braunbeck

Bodenseewerk Perkin-Elmer and Co., GmbH.
Überlingen – Bodensee.

[1] Fischer, K. A., and Brandes, G., *Naturwiss.*, **43**, 223 (1956).

Rotational Analysis of the $C^2\Pi - X^2\Sigma^+$ System of CaCl

Although many of the electronic band-systems of the monohalides of Group II*A* are readily observed in emission and absorption, little is known about the properties of these molecules, other than their force constants and the positions of their lower-lying electronic energy states. Only one complete rotational analysis, of the system $A^2\Pi_1 - X^2\Sigma^+$ in BeF has yet been given[1].

In many cases, the rotational structure is, through the near equality of B-values, too close to be resolved, but new photographs on a 6·65-m. grating spectrograph suggest that analyses of some systems will be possible. One such system is $C^2\Pi - X^2\Sigma^+$ in CaCl, which may conveniently be observed in absorption. We have been able to analyse the 0,0 band, and the shorter wave-length sub-bands of the 1,0 and 0,1 bands. The upper state is sufficiently close to Hund's case *a* for the two sub-bands to be treated independently to give effective values of B' and D'. The Λ-type doubling, which is negligibly small in the shorter wave-length sub-bands, but observable in the longer wave-length components, shows that the C state is $^2\Pi_{1reg.}$, presumably $\cdots(w\pi)^4(v\pi)$, $^2\Pi_r$. An earlier, incomplete analysis[2] of this system is incorrect. The constants obtained in the present analysis are summarized in Table 1.

Table 1. Summary of Constants for the $C^3\Pi_r - X^1\Sigma^+$ System of $Ca^{35}Cl$

State	T_e	ΔG_{v+1}	B_e	10^4	$10^7 D$	r_e, Å.
$C^3\Pi_{2,0}$	26,557·80	333·86	0·14268	7·47	1·0$_0$	2·516$_0$
$C^3\Pi_{1,0}$	26,481·82		0·14216		1·0$_0$	
$X^1\Sigma^+$	0	367·53	0·15156	7·8$_0$	1·0	2·439$_0$

It is of some interest to compare the internuclear distances in the ground-states of the monohalides with those found in the dihalides[2]. Figures are given in Table 2. They suggest that the distances in the monohalides are about 3 per cent shorter than in the corresponding dihalide.

Table 2. Internuclear Distances (refs. 1 and 3)

BeF_2	1·40 ± 0·03 Å.	BeF	1·361 Å.
$BeCl_2$	1·75 ± 0·02	BeCl	(1·7)
MgF_2	1·77 ± 0·02	MgF	1·75$_5$
CaF_2	2·10 ± 0·03	CaF	(2·02)
$CaCl_2$	2·51 ± 0·03	CaCl	2·439

E. Morgan
R. F. Barrow

Physical Chemistry Laboratory,
University,
Oxford.

[1] Herzberg, "Molecular Spectra and Molecular Structure", 1, "Spectra of Diatomic Molecules" (Van Nostrand, New York, 1950).
[2] Parker, *Phys. Rev.*, **47**, 349 (1935).
[3] Akishin, P. A., *et al.*, see Bastiansen and Lund, "Ann. Rev. Phys. Chem.", **10**, 43 (1959).

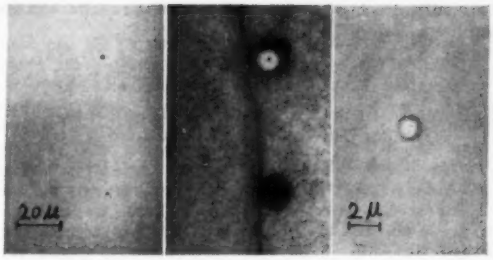
Dark Blue Ring developed by Electron Bombardment in Rock Salt

We have recently studied the effect of electron bombardment on rock salt crystal obtained from the Halle Company in Germany. It was found that the blue or colloidal coloration was produced on the surface of the specimen bombarded with electrons at high temperature[1]. In the course of the experiments we have also observed that the dark blue rings or circles were scattered on the coloured surface. In this communication we shall be concerned mainly with the behaviour of these rings or circles.

Bombardment was performed as follows. The cleaved specimen was annealed for 60 min. at elevated temperature *in vacuo* and then bombarded with electrons for 15 min. at the same temperature. The bombarding voltage and current density were 18 kV. and 80 μamp./cm.^{2} respectively.

Figs. 1*a* and *b* show the same field of vision of the specimen before and after bombardment, and Fig. 1*c* gives a magnified picture of the small point shown in Fig. 1*a*. These observations show that the rock salt as received contained a large number of small cubic cavities within the crystal (about 10^7/c.c.); and the centre of the dark blue ring corresponds to a cavity just beneath the surface and the centre of the circle to one which is somewhat deeper in the crystal. The edges of this cavity are parallel to the {100} planes of rock salt.

The changes in the ring pattern are shown in Fig. 2 as a function of temperature during the bombardment. The diameters of both outer and inner circles of the rings increased with the rise of temperature, and at the same time, the dimension of each cubic pit at the centre also increased. At temperatures above 370° C., only large cubic pits were produced.



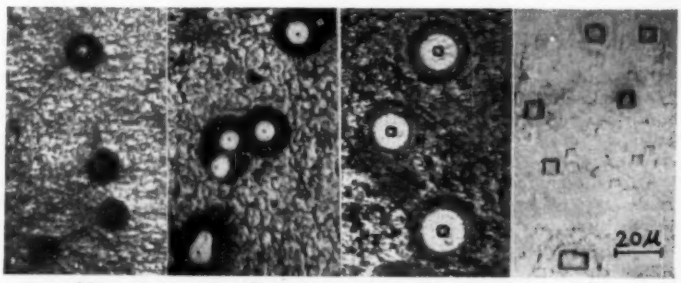
(*a*) (*b*) (*c*)

Fig. 1. Dark blue ring and circle developed by electron bombardment. (*a*) Before bombardment; (*b*) after bombardment (T = 300° C.); (*c*) magnified photograph of the small point in (*a*)

Further heat treatment of the ring after bombardment led to increase of the inner diameter with the rise of temperature, but the outer ring did not increase, and at about 450° C. it was bleached out entirely.

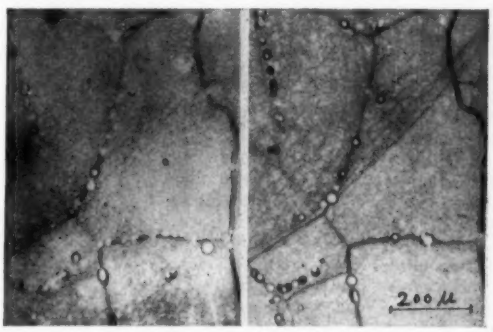
Apart from the dark blue ring or circle, we also observed the development of a coloured contour line as shown in Fig. 1*b*. Figs. 3*a* and *b* show matching surfaces of the cleaved specimen after bombardment; it can be seen that the matching of the contour lines was very good. Hence these contour lines are believed to indicate the sub-grain boundaries.

We are now investigating the mechanism of development of these peculiar types of coloration in



(*a*) (*b*) (*c*) (*d*)

Fig. 2. The change in the ring pattern as a function of temperature: (*a*) 290° C., (*b*) 320° C., (*c*) 360° C., (*d*) 380° C.



(*a*) (*b*)

Fig. 3. Matching surfaces of cleaved specimen after bombardment (T = 320° C.); (*b*) was reversed in printing

rock salt. A detailed discussion of the results will be published later.

S. Yoshida
T. Ikeda

Research Institute for Scientific Measurements,
Tohoku University, Sendai, Japan.

[1] Yoshida, S., and Ikeda, T., *J. Phys. Soc. Japan*, **14**, 473 (1959).

CRYSTALLOGRAPHY

Crystal Structure of Mellitic Acid

The only published crystallographic data for mellitic acid, to the best of our knowledge, are those reported by Deverin[1]. According to his observations mellitic acid is dimorphic: rhombohedral when crystallized from aqueous solution at room temperature, and of an unidentified lower symmetry class when separated from hot aqueous solution.

We obtained large prism-shaped crystals from a hot solution in 50 per cent nitric acid. The crystals were orthorhombic, optically negative, elongated in *b*-axis direction, pseudo-hexagonal, with well-developed forms {100}, {102}, {010}, exhibiting a perfect cleavage parallel to (010). The crystals were not stable for long periods, especially not when irradiated by X-rays, but became opaque and eventually disintegrated in a white crystalline powder. We assumed at first that the loss of nitric acid as solvent of crystallization might be the cause of the disintegration, but the micro-combustion analysis showed that our specimens had been pure mellitic acid. The phenomenon has been, therefore, explained as a transition from the orthorhombic high-temperature modification to another of low temperature, probably identical to the rhombohedral modification, observed by Deverin. It is rather interesting that our orthorhombic crystals, obtained from nitric acid solution, were considerably more stable while in contact with the mother liquor. Therefore, all our X-ray diffraction measurements were carried out with the specimen sealed over a drop of the mother liquor in a Lindeman glass capillary.

The unit cell and space group were determined from the oscillation and Weissenberg diffraction photographs taken with copper $K\alpha$-radiation. The orthorhombic unit cell, which has the dimensions: $a = 16{\cdot}60$ Å.; $b = 8{\cdot}17$ Å.; $c = 19{\cdot}17$ Å., contains eight molecules $C_{12}H_6O_{12}$. The space group is $C^{10}_{2h} - Pccn$. All possible $0kl$, $k0l$ and $hk0$ reflexions were recorded with the help of an integrating Weissenberg goniometer. The relative intensities were determined from the multiple film photographs with the use of a microdensitometer. The ratio of the weakest to the strongest intensity of 1 : 16,000 was obtained.

The Patterson projection on the (010) indicated: (i) that the molecules of mellitic acid formed layers parallel to the (010), (ii) that in the *b*-axis period two such mutually displaced layers were involved. The overlapping of these layers in the projection gave a vector map with an intricate hexagonal pattern which could not be interpreted satisfactorily. The arrangement of the molecules was then derived from the (100) and (001) Patterson projections. The molecules occupy positions on the diad axes so that the unit cell contains two symmetrically independent groups of four molecules which are defined in the International Tables[2] by the (*c*) and (*d*) sets of co-ordinates, respectively. The ambiguity in the orientation of the molecules toward the diad rotation axis was solved with the help of a trial-and-error procedure in which both the (010) vector map and the $h0l$ intensity data were considered. In this stage of the analysis it became evident that numerous possibilities for locating oxygen atoms would require tedious computational work of successive refinement. Therefore, a new direct method for the phase determination was elaborated by one of us[3] and applied to the sign determination of the structure factors $F(hk0)$ and $F(0kl)$. The electron density maps, computed on the basis of these signs, gave the first co-ordinates of oxygen and carbon atoms, which enabled an efficient refinement of the (010) electron density map, resulting in the complete elucidation of the structure. The final electron density map on the (010) is given in Fig. 1.

All carbon atoms of a molecule lie in a plane which is inclined to the (010) at not more than about two degrees. Each molecule is surrounded by six nearest molecules with the approaches in the direction of their carboxyl groups at the distance of $2{\cdot}62 \pm 0{\cdot}01$ Å. between oxygen atoms. This value for the oxygen separation between two carboxyls is evidence of a strong hydrogen bonding which links molecules together in an endless layer parallel to (010). A preliminary investigation by infra-red spectra confirmed roughly this hydrogen bond-length (Hadži, D., private communication). The next layer is derived in the same way but, in relation to the former, it is displaced along the *c*-axis by the amount of $z_2 - z_1 = 0{\cdot}151$, where z_1 and z_2 are the parameters of the centre of the two symmetrically independent molecules. The whole crystal structure of mellitic acid is thus built up by the alternation of these two layers with the separation of $1/2b$ between them (Fig. 2). Since the diad axis lies in the plane of the benzene ring and passes through two opposite carboxyl carbon atoms, the orientation of six carboxyls of one molecule is defined by four planes differently inclined to the plane of the benzene ring. The most inclined pair of carboxyls approaches the carboxyls of both upper and lower layer at the distance of 2·68 Å. between oxygen atoms. This approach may be accounted for by hydrogen bonding between layers. Such interlayer contacts occur three times less than the contacts within the layer, which explains the perfect cleavage parallel to the layer plane. Moreover, the contacts between

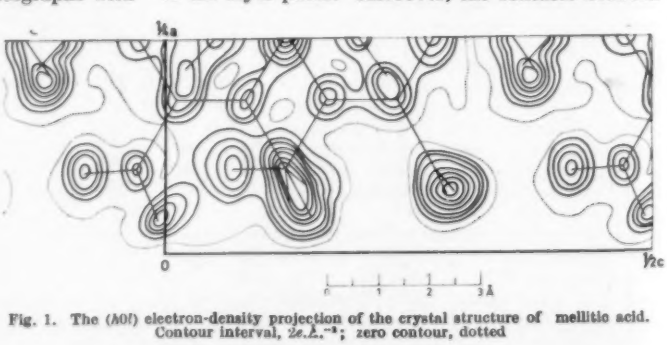


Fig. 1. The (*h*0*l*) electron-density projection of the crystal structure of mellitic acid. Contour interval, $2e.Å.^{-2}$; zero contour, dotted

carboxyls within the molecule itself occur at distances of 2·69, 2·71 and 2·75 Å. between oxygen atoms. Consequently, all oxygen atom contacts in the crystal structure are not essentially different, varying from 2·62 to 2·75 Å. If we assume, therefore, that the hydrogen bonding may be effectuated by all these contacts, then there are in the structure more hydrogen bonds than protons. This is possible only if a randomness of the proton positions is admitted. In favour of this assumption is also the symmetry of the carboxyls, since only one value of 1·23 ± 0·01 Å. for C–O distance has been found for all carboxyls and not only for those located on diad axis. The insignificant difference in length of the C–O bonds was previously established only for ionized carboxyls[4]. The symmetry of the carboxyl as well as a slightly shorter C–O bond found in the present case may be explained by the unique molecular structure of mellitic acid, which enables all carboxyls to be engaged in a hydrogen bond network throughout the crystal structure.

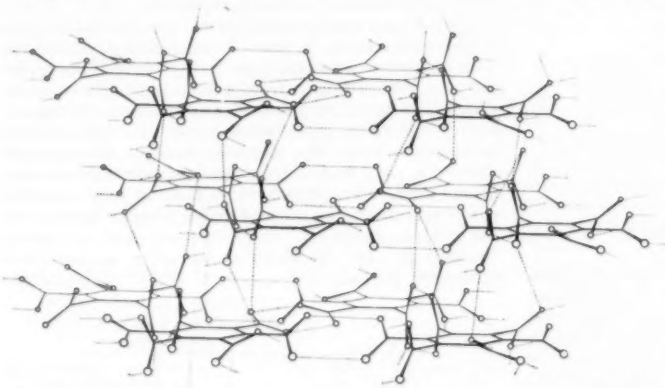


Fig. 2. The layers of the crystal structure of mellitic acid as viewed in the direction of the *a*-axis. The separation between layers is 1/2*b*. The hydrogen bonds between oxygen atoms (circles) are shown by dotted lines

The more accurate determination of the bond-lengths has been prevented by overlapping of the atoms in the electron density projection. The three-dimensional Fourier synthesis as well as the difference synthesis is therefore anticipated. The C–C bond-lengths within the benzene ring as well as from it to carboxyl, obtained from the projections, are in good agreement with the previously reported data for this kind of compound, that is, 1·40 ± 0·01 Å., and 1·50 ± 0·01 Å., respectively. The reliability indices are: $R(h0l) = 0·12$; $R(0kl) = R(hk0) = 0·13$.

We wish to thank Miss N. Katinčić for carrying out the synthesis of mellitic acid starting from xylene. We are also indebted to Mrs. I. Guštak-Mašek for the micro-analysis and to Mrs. G. Finžgar for valuable help in the computation.

A. Bezjak
D. Grdenić

Department of Structural and Inorganic Chemistry,
Ruder Bošković Institute,
54 Bijenička c., Zagreb, Yugoslavia.

[1] Deverin, L., *Bull. Soc. Vaud. Sci. Nat.* (Lausanne), **59**, 417 (1937).
[2] International Tables for X-Ray Crystallography, 1 (The Kynoch Press, Birmingham, 1952).
[3] Bezjak, A., *Acta Cryst.*, **12**, 765 (1959).
[4] van Bommel, A. J., and Bijvoet, J. M., *Acta Cryst.*, **11**, 61 (1958). Marsh, R. E., *ibid.*, **11**, 654 (1958). Hahn, T., and Buerger, M. J., *Z. Krist.*, **108**, 130, 419 (1957).

Thermal Expansion of Triglycine Sulphate

Matthias, Miller and Remeika[1] were the first to observe that triglycine sulphate becomes ferroelectric below 47° C. The dielectric properties and the specific heat of this crystal have been studied through the transition temperature by Hoshino, Mitsui, Jona and Pepinsky[2]. The observed variation of the dielectric properties as a function of temperature in this crystal shows that the transition is of second order. Hoshino *et al.* concluded that the anomaly is not of the λ-type, since their specific heat – temperature curve showed only a hump. It was decided to investigate the thermal expansion of this crystal as it might throw some light on the nature of the transition.

Triglycine sulphate belongs to the monoclinic system. Wood and Holden[3] determined the unit cell dimensions and the optical properties of this crystal. The complete structure of the ferroelectric phase of this crystal has been determined by Hoshino, Okaya and Pepinsky[4]. The crystallographic axial system of these authors is used in this communication in designating the directions in this crystal. The thermal expansion of triglycine sulphate and its temperature variation have now been investigated both above and below the transition point by the interferometric method for the following directions in the crystal: (1) parallel to the *b* axis, which is also the axis of spontaneous polarization in the crystal, and (2) parallel to the *a* and *c* axes, and perpendicular to the *a* axis in the (010) plane.

The specimens used for the expansion measurements along these four directions were cut from different crystals of triglycine sulphate and the measurements were repeated several times for each direction.

The variation of the thermal expansion coefficient (α) with temperature (T) for these four directions is plotted in Fig. 1. The results are summarized as follows:

Along the *c* and *a* axes and perpendicular to the *a* axis, the crystal expands as it is heated up to the transition point, the expansion coefficient becoming larger and larger as the transition point is approached. At the transition point there is an abrupt reversal of the sign of the expansion coefficients, the crystal contracting along these directions above the transition point. The behaviour along the *b* axis is just the opposite. The crystal contracts on heating up to the transition point and thereafter expands in this direction.

From the continuous change in the dimensions of the crystal, it appears that the transition is of the second order. This result is in agreement with that reported by Hoshino *et al.*[2]. From the nature of the α versus T curves (Fig. 1) one has to infer that the anomaly is of the λ-type, which does not agree with the observations of Hoshino *et al.*[2] on the specific heat.

Ubbelohde and Woodward[5] have found that in Rochelle salt the direction of maximum expansion coefficient lies almost along the direction of the shortest hydrogen bond. From the structural data of Hoshino, Okaya and Pepinsky[4] and the present

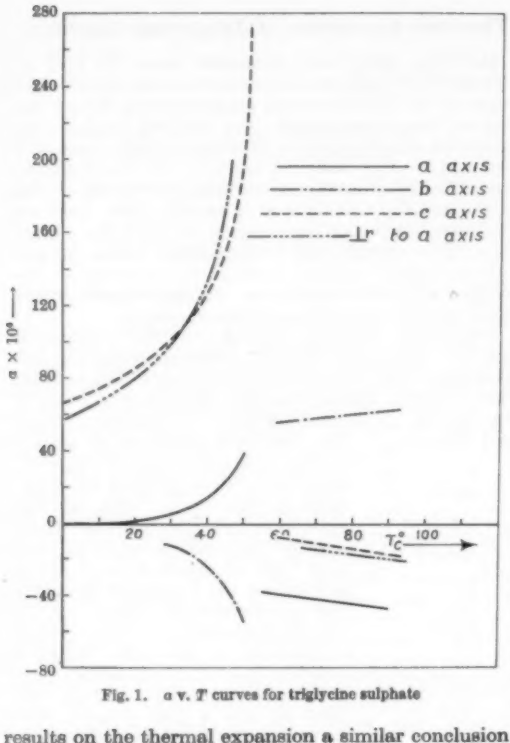


Fig. 1. α v. T curves for triglycine sulphate

results on the thermal expansion a similar conclusion could be drawn, namely, the direction of maximum expansion coefficient coincides nearly with the direction of the shortest hydrogen bond (length 2·438 Å.), which according to Hoshino *et al.*[4] is responsible for the dipole reversal mechanism.

The temperature at which the abrupt change in sign of α occurs has been observed to be slightly different for measurements along different directions, but all of them lie between 51° C. and 53° C., which is definitely higher than the value of the transition temperature of 47° C. reported by Matthias *et al.*[1] and Hoshino *et al.*[4]. It should be mentioned that all the measurements reported here were carried out while the crystals were heated. Taking measurements while the crystal was being cooled, it was found that the change in sign of α occurred at about 47° C. Preliminary evidence has also been obtained for thermal hysteresis in this crystal. Further investigations are under way to study the thermal hysteresis and a detailed report of the results will be published in due course.

I wish to express my thanks to Prof. R. S. Krishnan for his interest and to Dr. Vedam and Dr. Srinivasan for discussions. I also thank the former for suggesting the problem.

S. Ganesan

Department of Physics,
Indian Institute of Science,
Bangalore 12.
Dec. 10.

[1] Matthias, B. T., Miller, C. E., and Remeika, J. P., *Phys. Rev.*, **104**, 849 (1956).
[2] Hoshino, S., Mitsui, T., Jona, F., and Pepinsky, R., *Phys. Rev.*, **107**, 1255 (1957).
[3] Wood, E. A., and Holden, A. N., *Acta Cryst.*, **10**, 145 (1957).
[4] Hoshino, S., Okaya, Y., and Pepinsky, R., *Phys. Rev.*, **115**, 323 (1959).
[5] Ubbelohde, A. R., and Woodward, I., *Proc. Roy. Soc.*, A, **185**, 448 (1946).

CHEMISTRY

Recrystallization of Amorphous Copper Sulphate Monohydrate

When copper sulphate pentahydrate is dehydrated in a vacuum at room temperature, the resulting product, of composition copper sulphate monohydrate, possesses no evident crystal structure as shown by X-ray examination[1]. When the amorphous product is heated, crystallization occurs as shown by the appearance of the X-ray lines of the crystalline monohydrate[2].

The changes which occur on heating can also be followed by investigating the changes in the electron spin resonance spectrum, since the spectrum of the paramagnetic copper ion is influenced by its environment in the solid which, of course, will change as crystallization occurs. The spectra were recorded on a 'Varian Model 4500' spectrometer. The curves in Fig. 1 are traces of the derivative of the absorption curve as recorded by this instrument.

Copper sulphate pentahydrate was ground to pass a 200-mesh sieve. Curve F illustrates the electron spin resonance spectrum on this powdered material. Curve A was obtained from a portion of this material which had been dehydrated in a vacuum desiccator over magnesium perchlorate until the composition corresponded to the monohydrate. The sample showed a very weak broad absorption. The extreme broadening was presumably due to the random environment of the paramagnetic copper ions in the amorphous material. When the material is heated, changes in the spectrum occur after an apparent induction period. Curves B, C and D indicate the

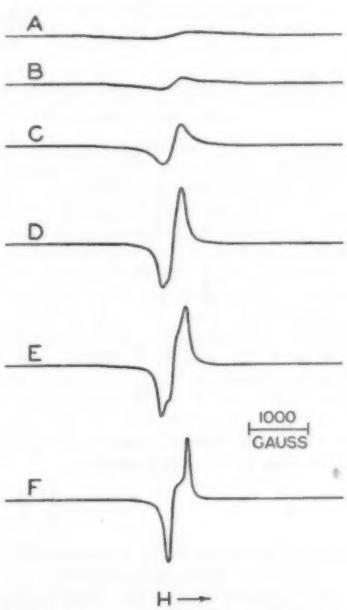


Fig. 1. Electron spin resonance spectra of copper sulphate hydrates. A, $CuSO_4.H_2O$ freshly prepared; B, $CuSO_4.H_2O$ heated 170 min. at 75° C.; C, $CuSO_4.H_2O$ heated 190 min. at 75° C.; D, $CuSO_4.H_2O$ heated 200 min. at 75° C.; E, Crystalline $CuSO_4.H_2O$ prepared at 110° C.; F, powdered $CuSO_4.5H_2O$.
Curves A, B, C and D were recorded at twice the amplification of curves E and F

traces obtained when the length of time of heat treatment of the sample at 75° C. was increased. With the recrystallization of the solid, the absorption narrows. Curve *E* is for a sample which was prepared and completely recrystallized at 110° C. A small amount of fine structure on the low-field side of the maximum is evident in curves *D* and *E*.

X-ray powder diagrams were taken at various stages of the recrystallization, using a General Electric recording X-ray diffraction unit. The material which gave the electron-spin resonance spectrum of curve *A* showed no diffraction lines. After heating, the X-ray diffraction lines of the monohydrate appeared.

It is interesting to note that the electron-spin resonance spectrum of a sample only partially recrystallized could not be simulated by the spectrum of a mixture of the amorphous monohydrate and the completely recrystallized compound. Even under higher amplification, the spectrum of sample *C* in Fig. 1 shows no evidence of fine structure on the low-field side of the maximum. A mixture of 19 per cent of the recrystallized monohydrate (sample *E*) and 81 per cent of the amorphous material (sample *A*), which gave the same overall intensity of absorption, very clearly showed fine structure. The very first stages of recrystallization do not appear to involve the formation of regions in the solid which yield the same electron-spin resonance spectrum as the completely recrystallized material of sample *E*.

The financial assistance of the National Research Council of Canada is gratefully acknowledged.

G. A. Specken
K. J. McCallum

Department of Chemistry,
University of Saskatchewan,
Saskatoon.

[1] Kohlschutter and Nitschmann, *Z. phys. Chem.*, Bodenstein Festband, 494 (1931).
[2] Frost, Moon and Tompkins, *Canad. J. Chem.*, **29**, 604 (1951).

Some Atomic Reactions by Absorption Spectroscopy

Norrish and co-workers[1-3] have shown that vibrationally excited oxygen molecules are produced by flash photolysis of chlorine dioxide, nitrogen dioxide and ozone. These molecules are believed to originate in reactions of the type:

$$O + ClO_2 \rightarrow ClO + O_2^* \qquad (1)$$

Combination of familiar atomic flame techniques[4,5] with absorption spectroscopy should enable such reactions to be studied directly. We have made observations of the reactions of atomic oxygen with ClO_2, NO_2 and O_2 in this way, at pressures of 0·1–30 mm. mercury. Similar studies have been made using atomic nitrogen and hydrogen in the cases of ClO_2 and O_2.

Atoms were produced from the molecular gas by electrodeless discharges (at 2,450 or 30 Mc./s.) and mixed with the reactant gas at one end of a reaction cell, 2 cm. in diameter and 30 cm. long, with quartz end-windows. Linear flow-rates through the cell were between 100 and 250 cm./s. The continuum from a high-pressure xenon source was focused through this cell, and viewed with a Hilger quartz, Littrow type, spectrograph. The extent to which atomic oxygen had reacted could be seen from the decrease in the green after-glow (caused by reaction of O with NO formed in the discharge from nitrogen impurities) when the reactant was added, and, in the case of atomic nitrogen, from the decrease in the Rayleigh after-glow. In most cases spectroscopic data were obtained at flow-rates such that these after-glows were almost extinguished, that is, at complete consumption of the atoms. Under these conditions weak flames were usually seen in the reaction vessel.

Some observations are summarized in Table 1.

Table 1. Spectral Observations of Some Atomic Flames

Atom	Reactant		
	ClO_2	NO_2	O_2
O	ClO_2 removed ClO produced Red flame	NO produced	Red flame (weak)
N	ClO_2 removed ClO produced (weakly) Green flame	—	O_2 removed No NO Blue-white flame
H	ClO_2 removed No ClO or OH Green flame (weak)	Red flame	O_2 removed No OH Red flame (weak)

The sensitivity of the spectroscopic system can be judged from the fact that amounts of NO and NO_2 giving partial pressures of 0·01 and 0·10 mm. mercury respectively could be observed readily. The rotational temperature of the NO produced in the NO_2 — O reaction was the same as the temperature of the reaction vessel. A tenfold increase in pressure by the addition of argon gave no new information. No evidence was found for the existence of vibrationally excited species.

A quantitative examination of the system is necessary to see what limitations might apply to the production and detection of vibrationally excited oxygen. By adding sufficient NO_2 to quench the after-glow in the products of the oxygen discharge[6], and making spectroscopic determinations of the NO molecules produced, the concentration of atomic oxygen was established to be 10 per cent of the gases from the discharge at 2 mm. mercury. Although the efficiency of O_2 in deactivating O_2^* is not known, one collision in 10^5 can be taken as reasonable in comparison with the efficiencies given by Norrish[3] for other molecules. If it is further assumed that all the O atoms form O_2^* by reaction (1) the total number of O_2^* molecules in the reaction vessel is calculated to be $3·6 \times 10^{16}$ at 2 mm. mercury, or 0·6 per cent of all molecules present.

An estimate of the amount of the other product of reaction (1) can also be made in the case of ClO, for which radicals the rate constant for recombination has been determined[7]. It gives a total number of ClO radicals of $2·7 \times 10^{16}$, or 0·4 per cent of the gas at 2 mm. mercury. Our failure to find O_2^*, while ClO was readily detected, may be due to: (*a*) a deactivation efficiency of O_2 greater than the estimated 1 in 10^5; (*b*) a smaller absorption coefficient for O_2^* than for ClO; or (*c*) O_2^* not being produced by reaction (1), which possibly requires metastable O atoms rather than the ground-state atoms given by our discharges. Similar reasoning can also be applied to the NO_2 — O reaction, except that now the NO produced, since it is stable, reaches 10 per cent of the total gas.

Concentrations of atomic nitrogen were measured by titrating the active nitrogen with nitric oxide[8], and afterwards determining the amounts of NO by spectral absorption when the discharge was extinguished. At 20 mm. mercury about 1 per cent nitrogen atoms were produced. The disappearance of ClO_2 by reaction with N atoms, but only very weak ClO, and no NO absorption can be interpreted by a low rate of:

$$N + ClO_2 \rightarrow NO + ClO \qquad (2)$$

or by rapid subsequent disappearance of the products of this reaction. Lack of NO absorption in the reaction of N with O_3 can be explained as follows. When [N] is greater than $[O_3]$, the nitric oxide formed is destroyed rapidly by the excess of free atoms. On the other hand, when ozone is in excess it will react rapidly with NO (ref. 9).

The concentration of atomic hydrogen was not measured, but previous experience[10] with the same type of discharge indicates a concentration of about 10 per cent. Again, the decrease in ClO_2 absorption, without any OH or ClO absorption, can be taken as evidence that the initial attack does not produce these radicals or that their subsequent reactions are extremely rapid.

The ease of detection of small quantities of NO and ClO suggests that absorption spectroscopy could be a useful tool for studying kinetics in steady-state flow systems of this type. Moreover, the spectroscopic measurement of NO has proved to be very useful in determining the concentrations of oxygen and nitrogen atoms. The visible reaction flames suggest that a study of their emission spectra may provide information regarding the initial steps in these reactions.

We are pleased to acknowledge the fellowship assistance from the U.S. National Science Foundation (H. P. B.) and from the Nuffield Foundation (H. I. S.).

H. P. Broida
H. I. Schiff
T. M. Sugden

Department of Physical Chemistry,
University of Cambridge. Jan. 9.

[1] Lipscomb, F. J., Norrish, R. G. W., and Thrush, B. A., *Proc. Roy. Soc.*, A, **233**, 455 (1956).
[2] McGrath, W. D., and Norrish, R. G. W., *Proc. Roy. Soc.*, A, **242**, 265 (1957).
[3] Norrish, R. G. W., *Proc. Chem. Soc.*, 247 (1958).
[4] Gaydon, A. G., and Wolfhard, H. G., *Proc. Roy. Soc.*, A, **213**, 366 (1952).
[5] Ferguson, R. E., and Broida, H. P., Fifth Symposium on Combustion, 754 (Reinhold Pub. Corp., New York, 1955).
[6] Elias, L., Ogryzlo, E. A., and Schiff, H. I., *Canad. J. Chem.*, **37**, 1680 (1959).
[7] Edgecombe, F. H. C., Norrish, R. G. W., and Thrush, B. A., *Chem. Soc. Spec. Pub.*, **9**, 121 (1958).
[8] Kaufman, F., *J. Chem. Phys.*, **28**, 992 (1958).
[9] Ford, H. W., Doyle, G. J., and Endow, N., *J. Chem. Phys.*, **26**, 1336 (1957).
[10] Shaw, T. M., "Formation and Trapping of Free Radicals", Chapter 3 (Academic Press, New York, 1960).

Ionic Hydration in Mixtures of Strong Acids and Water

From Raman and nuclear magnetic resonance measurements the concentrations of species present in mixtures of strong acids with water have been evaluated[1–3].

These measurements show that each ionic constituent has a maximal concentration at a certain composition of the mixture. The existence of such maxima can be understood in terms of hydration of the ionic species present.

Consider the following reaction:

$$HA + n\,H_2O \rightleftarrows H^+(H_2O)_{n_+} + A^-(H_2O)_{n_-} \qquad (1)$$

$$n_+ + n_- = n$$

Application of the law of mass action to (1) gives:

$$a_{H^+(aq.)} \cdot a_{A^-(aq.)} = K_n \cdot a_{HA} \cdot a_{H_2O}{}^n \qquad (2)$$

where $a_{H^+(aq.)}$ and $a_{A^-(aq.)}$ denote the activities of $H^+(H_2O)_{n_+}$ and $A^-(H_2O)_{n_-}$, and n is the total number of water molecules bound to the ions H^+ and A^-. By differentiation of (2) and combination with the Gibbs–Duhem equation, it can be shown that the activity product of the hydrated ions will have a maximum at the concentration[4]:

$$X^s_{HA} = \frac{1}{1+n} \qquad (3)$$

where X^s_{HA} is the stoichiometric mole fraction of acid. The maximum will thus appear at the stoichiometric concentration corresponding to the assumed composition of the hydrated ions.

When considering concentrations of ionic constituents, the variation of their activity coefficients will be neglected. From the electroneutrality condition:

$$C_{H^+(aq.)} = C_{A^-(aq.)} \qquad (4)$$

and (2) it can be shown that, within the uncertainty of the variation of the ionic activity coefficients, condition (3) is retained for the location in the maximal concentration of A^-.

The maxima can be considered to arise from the competition of various hydration reactions with the formation of HA molecules. It can be stated that the weaker the acid the more will the maximal ionic concentration be displaced in the direction of pure water. In Table 1 a comparison is made between experiments and equation 3.

Table 1. Comparison of Mole Fraction of Maximal Ionic Concentration with Equation 3

n	X^s_{HA} (3)	NO_3^-	X^s_{HA} exp. ClO_4^-	HSO_4^-
1	0·50		0·40–0·50 (ref. 2)	0·42 (ref. 3)
2	0·33			
3	0·25	0·26 (ref. 5)		
4	0·20	0·21 (ref. 2)		

As seen from Table 1, the maximum ionic concentration of NO_3^- appears in the range where there are 3–4 H_2O per H^+, NO_3^-, while maximal concentration of ClO_4^- and HSO_4^- are in the range where there is about 1 H_2O per H^+, A^-. That nitric acid behaves differently from the other two acids is due to its weaker dissociation. From the data of Redlich *et al.*[2] it is found that even at $X^s_{HNO_3} = 0{\cdot}40$ there are more than four water molecules per mole of dissociated acid.

In the primary dissociation of H_2SO_4, the main hydrated ionic constituent is likely to be H_3O^+ (ref. 6). However, the displacement of the maximal ionic concentration from 0·50 to 0·42 implies that hydrated species other than H_3O^+ are also present. This further hydration can be taken into account. By assuming the formation of all possible hydrated ionic species, it can be shown that maximum concentration of the anion A^- will be located at $X^s_{HA} = 1/(1+\bar{n}')$, where $\bar{n}'$ approximates the average number of water molecules bound to the ions H^+ and A^- (ref. 4). For $X^s_{H_2SO_4} = 0{\cdot}42$ this gives $\bar{n}' = 1{\cdot}40$. The same value for $\bar{n}'$ was obtained by an approximate method of finding $\bar{n}'$ reported elsewhere[6]. Using the values of $\bar{n}'$ given in ref. 6, it is also found that at $X^s_{H_2SO_4} = 0{\cdot}42$ the free water practically disappears and becomes tied up as water of hydration. Now, Young *et al.*[3] have found that measurable amounts of sulphuric acid

start to appear at this same concentration. It thus seems that molecules of sulphuric acid cannot appear until nearly all free water has been consumed.

ERIK HÖGFELDT

Department of Inorganic Chemistry,
Royal Institute of Technology,
Stockholm 70.

[1] Chedin, J., *J. Chim. Phys.*, **49**, 109 (1952).
[2] Hood, G. C., Redlich, O., and Reilly, C. A., *J. Chem. Phys.*, **22**, 2067 (1954).
[3] Young, T. F., Maranville, L. F., and Smith, M. M., in Hamer, W. J., "The Structure of Electrolytic Solutions", 35 (John Wiley, New York, 1959).
[4] Högfeldt, E., *Acta Chem. Scand.* (in the press).
[5] McKay, H. A. C., *Trans. Farad. Soc.*, **52**, 1568 (1956) (quoting measurements by Young and Krawetz).
[6] Högfeldt, E., *Acta Chem. Scand.* (in the press).

The Sodium Silicate – Hydrogen Peroxide System

THE chemistry of silicic acid in solution is rather ill-defined[1] owing to the extremely weak nature of this acid[2] and its tendency to polymerize[3,4]. As there are many instances where hydrogen peroxide effectively prevents anionic polymerization[5,6], an attempt was made to investigate the hydrolysis of silicate ions in the presence of hydrogen peroxide by means of accurate conductivity[7] and *p*H measurements. For comparison, the same technique was applied to an identical series of solutions in the absence of hydrogen peroxide. Fig. 1 shows the results of the investigation on solutions which were 0·01 *M* in sodium silicate and, in the case of the 'peroxidized' system, approximately 0·1 *M* in hydrogen peroxide; sulphuric acid was 0·498 *M*.

The *p*H curve of the system sodium silicate–water (full lines) shows two changes: a weak one on addition of 4·75 ml. sulphuric acid and a strong change on addition of 9·3 ml. acid. The former change may be due to the reaction of the dihydroxy–*ortho*silicate ion[1-3] with hydrogen ions:

$$(SiO_2(OH)_2)^{2-} + H_3O^+ = (SiO(OH)_3)^- + H_2O \quad (1)$$

The latter change denotes obviously the completion of the neutralization reaction:

$$1OH^- + H_3O^+ = 2H_2O \quad (2)$$

the hydroxyl ions being those hydrolytically produced by sodium silicate. Corresponding changes in the conductivity curve of the same system are brought out by the interpolated point of intersection[8] *B* (equation 1) and point *D* (equation 2) respectively. The strong *p*H change (equation 2) seems to mask the final decomposition of the trihydroxy–*ortho*-silicate ion to silicic acid, although some indication of the latter reaction is borne out by the shape of the conductivity curve near point *D*.

A similar picture emerges from the investigation of the sodium silicate – hydrogen peroxide system (dotted lines), with the difference that as the *p*H of the initial solution lies below 10·75 (owing to the consumption of hydroxyl ions by hydrogen peroxide in alkaline solution) the points *A* and *B* are absent. A comparison of the specific conductivities in the two systems for solutions containing 6–9 ml. sulphuric acid reveals the rather significant finding of higher conductivity in the 'peroxidized' system (*p*H 8–10) which may be attributed to the lack of polymerization of the silicate ion in this system. The suggestion is therefore made that the study of the hydrolysis of the silicate ions is rendered less complex in the presence of hydrogen peroxide.

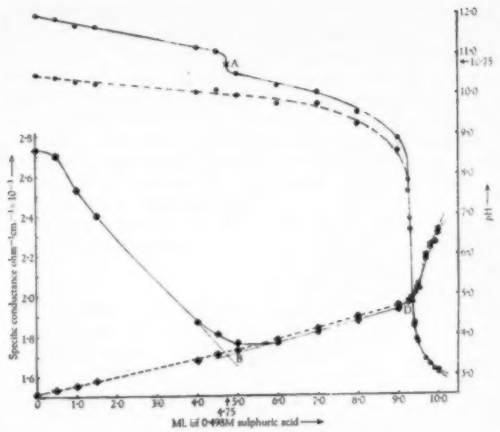


Fig. 1. Measurements of specific conductivity (◆) and *p*H (●) for the systems sodium silicate – hydrogen peroxide – water (dotted lines) and sodium silicate – water (full lines)

Detailed results of our investigation will be published elsewhere. We wish to acknowledge a research grant from Joseph Crosfield and Sons, Ltd., and the gift of pure unstabilized hydrogen peroxide from Laporte Chemicals, Ltd.

G. KAKABADSE
J. W. DEWSNAP

Department of Chemistry,
College of Science and Technology,
Manchester 1.

[1] Greenberg, S. A., *J. Chem. Educ.*, **36**, 218 (1959).
[2] Remy, H., "Treatise on Inorganic Chemistry", **1**, 493 (Elsevier, Amsterdam, 1956).
[3] Thilo, E., *Angew. Chem.*, **63**, 201 (1951).
[4] Alexander, G. B., *J. Amer. Chem. Soc.*, **76**, 2094 (1954).
[5] Kakabadse, G., and Wilson, H. J., *Nature*, **180**, 861 (1957).
[6] Jahr, K. F., "Flat Review of German Science 1936–1946", Pt. 3, 172.
[7] Constable, F. H., and Tegul, S., *Nature*, **160**, 55 (1947).
[8] Kortüm, G., and Bockris, J. O'M., "Textbook of Electrochemistry", **1**, 216 (Elsevier, London, 1951).

Volatile Organic Matter in Algal Culture Media and Sea Water

DURING an investigation of the extracellular products of marine algae, in which filtered culture media were concentrated by vacuum distillation, the distillates, which had an appreciable odour, were found to have a greater absorption of ultra-violet light than distilled water. The absorption of distillates increased by distilling at atmospheric pressure with ice cooling, a spray trap being incorporated in the apparatus. Examples of spectra from filtrates of uni-algal cultures of *Enteromorpha* sp., *Phaeocystis pouchetii* and *Phaeodactylum tricornutum*, bacteria-free *Ectocarpus confervoides* and membrane-filtered sea water are shown in Fig. 1. The overall shape of the absorption curves is somewhat affected by rate of boiling, degree of cooling, alkalinity of sample and freshness of the distillate. This suggests that more than one constituent may be present.

By redistilling distillates under various conditions it is possible to estimate that the recovery of volatile ultra-violet absorbent material is about 50 per cent when a distillate of 250 ml. is collected from a sample of 2·5 l. Measurements with CH_3COOH, C_2H_5COOH,

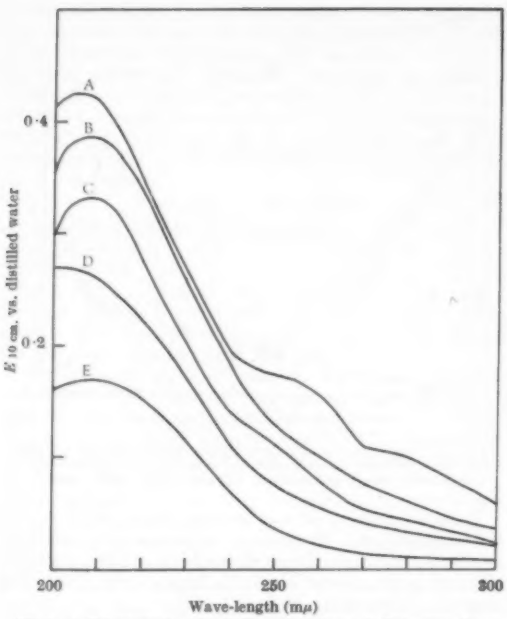


Fig. 1. *A*, *Ectocarpus* (× ½); *B*, *Phaeodactylum*; *C*, *Enteromorpha*; *D*, *Phaeocystis*; *E*, sea water

$CH_2OHCOOH$, H_2BO_3, $HCHO$, CH_3CHO, $(CH_3)_2CO$, $(CH_3)(C_2H_5)CO$, NH_3, CH_3NH_2, $(CH_3)_2NH$, $C_3H_7NH_2$ have shown that the absorptions found can only be accounted for if these substances are present (singly or as a mixture) at concentrations exceeding about 10 mgm./l. in the sample. Formic acid or trimethylamine at concentrations of about 1 mgm./l. could account for the ultra-violet absorption; but the former is unlikely to be volatile from sea water and the latter would necessitate larger carbon values than were actually found.

That the volatile material is at least partly organic is shown by carbon estimations carried out as follows. The distillates were boiled, after the addition of sodium hydroxide to retain carbon dioxide, and the vapours passed with a stream of oxygen through a simple 'empty tube' combustion train. The redistillation with alkali causes the loss or retention in the flask of nearly 50 per cent of the ultra-violet absorptive material. This heavy loss suggests that in fact the carbon content of the volatile material may be a good deal higher than the figures in Table 1 indicate.

Table 1. Carbon and Sulphur Determinations in Filtered Culture Media and in Sea Water

	Carbon (μgm./l.)	Sulphur (μgm./l.)
Ectocarpus confervoides (bacteria-free)		
Cultures I and II	—	36 and 33
Culture III	110	22
Enteromorpha sp. (uni-algal)	70	24
Phaeocystis pouchetii (uni-algal)	70, 100, 100	4·0, 6·0
Phaeodactylum tricornutum (uni-algal)		
Culture *A*	80	11, 9·5
Culture *B*	10, 50	2·5, 4·0, 3·0
Sea water, Lat. 50° 02′ N., Long. 4° 22′ W. (English Channel)		
Sept. 8, 1959	—	1·0
Oct. 13, 1959	—	1·5
Nov. 11, 1959	40, 40, 50	2·5, 2·0, 3·0, 2·0
Dec. 1, 1959	30, 40, 20, 30	1·5, 2·5, 3·5, 4·0

The characteristically 'weedy' odour of the distillates suggested the presence of organic sulphides since Haas[1] has shown that these substances are released from some marine algae. Micro-estimations of sulphur were carried out as follows. The distillates were passed through a small column packed with sulphur-free sugar charcoal, which had been found to remove odour and ultra-violet absorbent material. The absorbed material was volatilized at 600° C. in a stream of hydrogen and passed through a tube furnace at 1,000° C. to convert sulphur compounds to hydrogen sulphide[2]. This was then determined colorimetrically by the 'Lauth's violet' method of Polson and Strickland[3]. Sulphur contents of 1–4 μgm. sulphur/l. were found in samples of offshore sea water and higher values in cultures (see Table 1). Hydrogen sulphide could not be detected in the distillates (less than 0·1 μgm. sulphur/l. in the samples). If the sulphur were present as dimethyl sulphide the quantities found are quite insufficient to account for all the ultra-violet absorption. Further, the carbon : sulphur ratio is greater than in this compound. The nature of the other carbon compounds which must be present is as yet unknown.

These volatile materials were present in the media from bacteria-free cultures of *Ectocarpus* and appear to be released by the alga. Also, since they can be detected early in the growth of cultures of *Ectocarpus*, *Phaeocystis* and *Phaeodactylum* they are probably produced during healthy growth.

Fogg and Boalch[4] have already shown the presence of considerable quantities of extracellular nitrogenous matter in *Ectocarpus* cultures, and have suggested that it may have importance as a chelating agent. If the volatile sulphur is present as dimethyl sulphide or related compounds (which are known to form complexes with heavy metal salts[5]), the metal-binding properties of the extracellular products may be enhanced and may partly account for the marked resistance of *Ectocarpus* to compositions containing copper and mercury[6].

F. A. J. Armstrong
G. T. Boalch*

Marine Biological Association,
Plymouth.

* International Paints Research Fellow.

[1] Haas, P., *Biochem. J.*, **29**, 1297 (1935).
[2] Fogo, J. K., and Popowsky, M., *Anal. Chem.*, **21**, 734 (1949).
[3] Polson, D. S. C., and Strickland, J. D. H., *Anal. Chim. Acta*, **6**, 452 (1952).
[4] Fogg, G. E., and Boalch, G. T., *Nature*, **181**, 789 (1958).
[5] Challenger, F., "Aspects of the Organic Chemistry of Sulphur" (Butterworth, London, 1959).
[6] Harris, J. E., *J. Iron and Steel Inst.*, **148**, 405 (1943).

Some Phenolic Constituents of Cigarette Smoke

Phenol and a number of substituted phenols have strong tumour-promoting properties[1], and similar activity has recently been demonstrated in a phenolic fraction of cigarette smoke (Salaman, M. H., and Roe, F. J. C., private communication). It seemed of interest, therefore, to record some results we have obtained in an examination of phenols from the smoke of a variety of British cigarettes.

The cigarettes were smoked mechanically (2 sec. puff of 25 c.c. every 15 sec.) and the smoke was condensed in traps at −60°. Volatile material was

removed from the condensate by co-distillation with methanol, and the remaining tar, in methanol, was cooled to 0° to precipitate waxes. The filtered solution, in ether, was extracted successively with dilute aqueous acetic acid, sodium bicarbonate and sodium hydroxide, and the material recovered from the sodium hydroxide extract was methylated with diazomethane. Carboxylic acid esters present in the crude methylation product were hydrolysed with boiling ethanolic potassium hydroxide and the phenol ethers distilled. The compounds listed in Table 1 were obtained from a fraction b.p. 150–300° (1·1 gm. from smoke of 5,000 cigarettes) by gas-liquid chromatography[2]. They were identified by preparation of crystalline derivatives which were compared with authentic specimens and, where possible, by infra-red and ultra-violet absorption measurements. The cresyl ethers were eluted together from the chromatogram; the free cresols, however, were easily separated by counter-current distribution between benzene and a phosphate buffer as described by Golumbic[3]. The methoxyacetophenones obtained from the chromatogram were contaminated with unidentified material but this was removed by crystallization of the 2 : 4-dinitrophenylhydrazones.

The cigarette smoke must have contained the parent phenols of the ethers listed in Table 1 (veratrole, of course, could have arisen from either catechol or guaiacol). Some of the phenols have already been detected in cigarette smoke by spectroscopic and other means[2,4,5], and the isolation of phenol, *m*-cresol and guaiacol as their 3 : 5-dinitrobenzoates from smoke of Japanese cigarettes was reported[5] after the completion of the present work. The hydroxyacetophenones, however, have not previously been found in cigarette smoke. It is of interest, with regard to the promoting activity of the phenolic fraction of cigarette smoke (Salaman, M. H., and Roe, F. J. C., private communication), that phenol itself and the three cresols have all been shown to have tumour-promoting properties.

Results closely similar to the above were obtained by Mr. G. W. J. Matthews in this Laboratory by gas-liquid chromatography of phenol ethers derived from smoke of a brand of Argentinian cigarette said to contain fermented tobacco.

Table 1. Gas-Liquid Chromatographic Separation of Phenol Ethers derived from Cigarette Smoke

Peak No.	Compound	Estimated mgm. in smoke from 5,000 cigarettes	Crystalline derivative prepared
1	Anisole	40	2 : 4-dibromoanisole, m.p. 60°
2	*o*-Cresyl methyl ether *m*-Cresyl methyl ether *p*-Cresyl methyl ether	100	*o*-cresyl-3 : 5-dinitrobenzoate, m.p. 137° *m*-cresyl-α-naphthylurethane, m.p. 126° *p*-cresylbenzoate, m.p. 70°
3	Veratrole	200	4-nitroveratrole, m.p. 95–96° 4 : 5-dinitroveratrole, m.p. 132°
4	Unidentified	120	
5	*m*-Methoxyacetophenone + unidentified	(10)*	2 : 4-dinitrophenylhydrazone, m.p. 189–190°
6	*p*-Methoxyacetophenone + unidentified	(5)*	2 : 4-dinitrophenylhydrazone, m.p. 229–230°

Column 6 ft. × 1 in., 20 per cent silicone grease *E* 301 on 80–100 mesh acid-washed 'Celite'; nitrogen flow-rate, 100 ml./min. Temperature was not measured, but was arranged so that anisole was eluted in about 10 min. Fractions were collected in traps at − 60° C.
* Estimated from weights of 2 : 4-dinitrophenylhydrazones.

Simple phenols have been found in cured tobacco leaf but they appear to be present in very small amounts[6]. The alkali-soluble fraction of an alcoholic extract of our cigarette tobacco afforded, after methylation, a significantly smaller amount of phenol ethers with boiling points below 300° C. than we obtained from the cigarette smoke, and it seems very likely that at least a proportion of the phenols in the smoke is produced by pyrolytic decomposition of other tobacco constituents.

We are indebted to Dr. J. W. Cook for his interest in this investigation.

W. Carruthers
R. A. W. Johnstone
Medical Research Council
Carcinogenic Substances Research Group,
Washington Singer Laboratories,
University of Exeter.

[1] Boutwell, R. K., and Bosch, D. K., *Cancer Res.*, **19**, 413 (1959). Salaman, M. H., and Glendinning, O. M., *Brit. J. Cancer*, **11**, 434 (1957).
[2] Carruthers, W., Johnstone, R. A. W., and Plimmer, J. R., *Chem. and Indust.*, 331 (1958).
[3] Golumbic, C., *J. Amer. Chem. Soc.*, **71**, 2627 (1949).
[4] Rayburn, C. H., Harlan, W. R., and Hanmer, H. R., *Anal. Chem.*, **25**, 1419 (1953). Commins, B. T., and Lindsey, A. J., *Brit. J. Cancer*, **10**, 504 (1956). Bonnet, J., and Neukomm, S., *Helv. Chim. Acta*, **40**, 717 (1957). Wynder, E. L., and Wright, G., *Cancer*, **10**, 255 (1957). Clemo, G. R., *Tetrahedron*, **3**, 168 (1958).
[5] Izawa, M., Kobashi, Y., and Sakaguchi, S., *Bull. Agric. Chem. Soc. Japan*, **23**, 194 (1959).
[6] Onishi, I., and Yamamoto, K., *Bull. Agric. Chem. Soc. Japan*, **20**, 61, 70 (1956); **21**, 90, 181 (1957). Dymicky, M., and Stedman, R. L., *Tobacco Sci.*, **3**, 60 (1959).

BIOCHEMISTRY

Effect of Sulphydryl Group Reagents on Tryptophanase Activity

Both Gunsalus *et al.*[1] and Wada *et al.*[2] have reported that the presence of glutathione is advantageous during the assay of tryptophanase preparations. Wada *et al.* have also reported studies on the inhibition of tryptophanase by a number of SH-group reagents, and have suggested that the group is involved in the linkage between apoenzyme and substrate[3]. When we compare results which we have obtained at intervals since 1942, there is much agreement between us, but divergences, which may or may not be significant, do exist. Evans, Handley and Happold[4] reported a 12–20 per cent reduction of activity in washed cell preparation with sodium iodoacetate in a final concentration of $M/33$, and in later work with cell-free preparations activities were reduced by 20–25 per cent at concentrations of 5×10^{-3} M. At lower concentrations 5×10^{-5} and 10^{-6} M there has been no inactivation or even from time to time an apparent increase in the activity of the preparations. Further studies on inhibition were reported by Dawes and Happold[5]. Such preparations of tryptophanase are not necessarily free from other enzymes associated with pyridoxal phosphate-linked enzyme systems nor from the system which produced pyridoxal phosphate from pyridoxamine phosphate which Beechey and Happold described as a transaminase and which we now know contains a pyridoxamine phosphate oxidase similar to that found by Pogell in rabbit liver preparations; these divergences may be significant. During work in these laboratories on the purification of tryptophanase, the inhibition of activity by the SH-group alkylating reagent chloroacetophenone, and the reversal of SH-group inactivation, have been studied.

Table 1. Effect of Chloroacetophenone Concentration on Tryptophanase Activity

Chloroacetophenone concentration (M)	Tryptophanase activity	
	μgm. indole/5 min.	Percentage
0	25·5	100
$0·2 \times 10^{-4}$	25·5	100
$0·2 \times 10^{-4}$	24·3	95
$0·4 \times 10^{-4}$	14·3	56
$0·2 \times 10^{-4}$	6·9	27
$0·2 \times 10^{-3}$	0	0

The enzyme preparations used were obtained from *Escherichia coli*, and the tryptophanase activity was measured, as described by Beechey and Happold[6]. Reaction mixtures contained 0·5 ml. each of enzyme preparation (approximately 0·5 mgm.) protein; phosphate buffer (0·1 M, *p*H 7·8); potassium chloride solution (0·1 M); pyridoxal phosphate (100 μgm.); D,L-tryptophan ($1·96 \times 10^{-2}$ M, for L-form); and additions to 3·0 ml. Under these conditions at 37°, the rate of reaction was linear during the first 5 min., after which time 0·5 ml. of 10 per cent trichloracetic acid was used to stop the reaction. The indole produced was measured as previously described[6]. Pre-incubation with various reagents was carried out using reaction mixtures complete except for the addition of substrate.

Preliminary studies showed that chloroacetophenone at a final concentration of $0·2 \times 10^{-3}$ M required 20–30 min. pre-incubation for maximum inhibition. Using a 30-min. pre-incubation time, the effect of concentration of chloroacetophenone is shown in Table 1.

The effect of glutathione, at various concentrations, on the inhibition of tryptophanase activity by chloroacetophenone is shown in Table 2.

Table 2. Effect of Glutathione on Chloroacetophenone Inhibition of Tryptophanase

Glutathione concentration (M)	Tryptophanase activity (μgm. indole/5 min.), at various chloroacetophenone concentrations (M)				
	$0·2 \times 10^{-3}$	$0·4 \times 10^{-4}$	$0·2 \times 10^{-4}$	$0·4 \times 10^{-5}$	0
0	0·4	9·2	15·5	26·8	30·0
$0·2 \times 10^{-3}$	4·5	25·3	26·1	32·5	33·3
$0·2 \times 10^{-2}$	24·0	25·0	29·6	33·3	33·0

Glutathione was pre-incubated with enzyme for 60 min., after which chloroacetophenone was added and the mixture incubated for a further 30 min.

It can be seen that not only does glutathione protect tryptophanase from inhibition by chloroacetophenone, but also that it sometimes increases the activity of the preparation where no chloroacetophenone is present.

Further experiments showed that the extent of the increase in tryptophanase activity due to glutathione depended on the source and storage history of the preparation used. Maximum activation was found to occur at a glutathione concentration of $0·2 \times 10^{-3}$ M. When glutathione was replaced by cysteine in these experiments, initial results suggested progressive inhibition rather than activation. It was observed, however, that the yellow colour of reaction mixtures, due to pyridoxal phosphate, rapidly disappeared after the addition of cysteine. When pre-incubation of enzyme with cysteine was followed by the addition of co-factor only 5 min. before the addition of substrate, an increase in tryptophanase activity occurred. Here again, however, the extent of activation was variable, depending on the history of the enzyme preparation. Representative results are given in Table 3.

Table 3. Effect of Pre-incubation Time on the Activation of Tryptophanase by Cysteine

Pre-incubation time (min.)	Tryptophanase activity	
	μgm. indole/5 min.	Percentage of control
99·5	17·5	400
37·5	7·9	184
20·5	6·4	149
6·5	6·1	142
Control (no cysteine)	4·3	100

Pyridoxal phosphate was added at the end of pre-incubation, 5 min. before addition of substrate. Cysteine concentration was $0·2 \times 10^{-3}$ M.

It thus appears that cysteine enhances tryptophanase activity, presumably by reduction of oxidized SH-groups, but that this activation is masked by the removal of coenzyme, possibly by Schiff base formation. As the removal of coenzyme leads to the irreversible destruction of tryptophanase activity in the absence of metal ions, it appears that glutathione rather than cysteine should be used to protect the integrity of SH-groups during the purification of tryptophanase.

When sodium *p*-chloro mercuric-benzoate was used as an SH-group reagent inhibition showed itself at concentrations of 8×10^{-7} M and was almost absolute at 8×10^{-4} M. The tryptophanase activity was more than restored with 8×10^{-4} M glutathione.

Further work, which is continuing in these laboratories, indicates that cysteine protects tryptophanase from inhibition by chloroacetophenone. The reducing agent ascorbic acid has been found to inhibit tryptophanase activity under all conditions, but the reason for this remains obscure.

We wish to thank Miss Barbara Clough for technical assistance.

J. M. Turner
Frank C. Happold

Department of Biochemistry,
University of Leeds.

[1] Gunsalus, I. C., Galeener, C. C., and Stamer, J. R., "Methods in Enzymology", 2, 238 (Acad. Press, 1955).
[2] Wada, H., Morisue, T., Sakamoto, Y., and Ichihara, K., *J. Vitaminol.* (Japan), 3, 183 (1957).
[3] Wada, H., *et al.*, Proc. Internat. Symp. on Enzym. Chem., Tokyo and Kyoto, 1957 (Pergamon Press, 1958).
[4] Evans, W. C., Handley, W. R. C., and Happold, F. C., *Biochem. J.*, 36, 311 (1942).
[5] Dawes, E. A., and Happold, F. C., *Biochem. J.*, 44, 349 (1949).
[6] Beechey, R. B., and Happold, F. C., *Biochem. J.*, 66, 520 (1957).

Antigenicity of Œstrone–Protein Conjugates

The purpose of the present study was to determine whether steroids, when conjugated by stable covalent bonds to proteins, could act as haptenic groups in inducing steroid-specific antibodies. A detailed account of the method used for synthesizing œstrone–protein conjugates has been published elsewhere[1]. In brief, œstrone was converted to œstrone-17-*iso*cyanate and the latter was allowed to react with human and bovine serum albumin to yield the corresponding œstrone-17-carbamido-protein conjugates.

Rabbits were immunized with the œstrone–human serum albumin conjugate and the presence of precipitating antibodies in the sera was shown by the ring test. Two types of antibody were detected. One type was precipitated with œstrone–bovine serum albumin conjugate but not with bovine serum albumin and the reaction was inhibited by œstrone-17-carbamido-glycine. The other type was precipitated with human serum albumin but this

reaction could not be inhibited with the glycine derivative of œstrone.

These results demonstrate that specific antibodies were produced to (1) the œstrone residue and (2) the protein carrier. The production of a third type of antibody, directed to the carbamido linkage itself, was ruled out since no reaction was obtained with butyl–carbamido–bovine serum albumin conjugate.

These conclusions were confirmed by three other methods.

(1) Dale–Schultz[3]: Guinea pigs were passively sensitized with rabbit antiserum produced to the œstrone-human serum albumin conjugate and their uteri were mounted in a Dale–Schultz apparatus. Addition to the bath of œstrone–bovine serum albumin conjugate and of human serum albumin resulted in the usual uterine contraction (Fig. 1*a*). However, addition of bovine serum albumin was without effect (Fig. 1*b*). Prior incubation of the uteri with the glycine derivative of œstrone completely inhibited the contraction due to the conjugate but not to human serum albumin (Fig. 1*c*).

(2) B.D.B.–Hæmagglutination: The œstrone–bovine serum albumin conjugate and human serum albumin were coupled to rabbit erythrocytes via *bis*-diazotized-benzidine[3,4]. The sensitized erythrocytes were added to serial dilutions of rabbit antiserum to œstrone–human serum albumin conjugate with and without various test inhibitors. Œstrone–bovine serum albumin conjugate as well as the glycine derivative of œstrone strongly inhibited hæmagglutination of erythrocytes sensitized with the conjugate while testosterone-17-glucoside and 17-hydroxycorticosterone-21-sodium succinate exhibited lower inhibiting activity. On the other hand, none of the test inhibitors mentioned had any effect on hæmagglutination of the erythrocytes sensitized with human serum albumin.

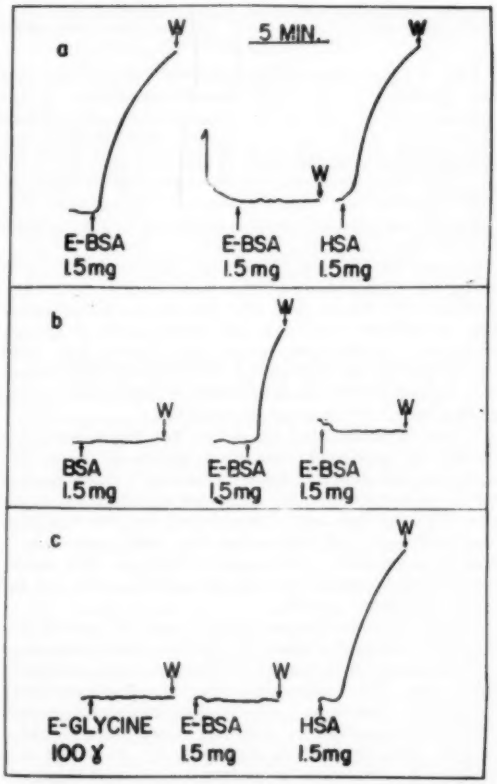


Fig. 1. *W*, Washing with Tyrode solution; *E-BSA*, œstrone–bovine serum albumin conjugate; *HSA*, human serum albumin; *BSA*, bovine serum albumin; *E-GLYCINE*, œstrone-17-carbamido–glycine

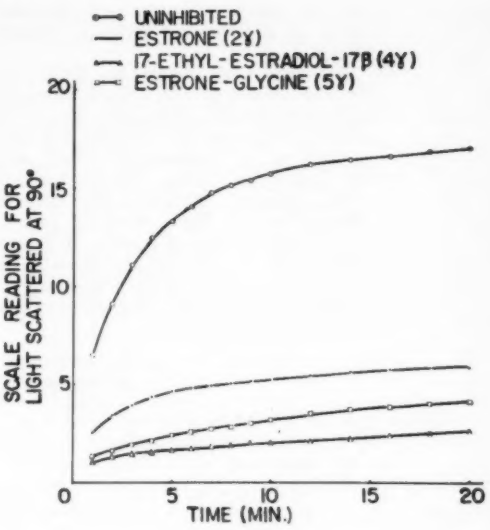


Fig. 2

(3) Turbidimetry: The course of the reaction between the conjugate of œstrone with bovine serum albumin and the rabbit antiserum to œstrone–human serum albumin conjugate was followed turbidimetrically by measuring light scattered at 90° with a Beckman *DU* spectrophotometer (cf. ref. 5). The curves obtained for the reaction at optimal antigen–antibody concentrations in the absence and in the presence of œstrone, the glycine derivative of œstrone and 17-ethynyl-œstradiol-17β are shown in Fig. 2. It can be seen that the steroids caused a marked reduction in turbidity; the percentage reduction in turbidity, due to these and other structurally related compounds, is given in Table 1.

The results presented demonstrate that the œstrone residue acts as a hapten when coupled to protein, and that the antibody site complementary to the œstrone residue is capable of accommodating not only œstrone but other structurally related molecules as well. These findings confirm in essence the conclusions of Beiser, Erlanger, Agate and Lieberman[6],

Table 1

Inhibitor	Amount added (γ)	Inhibition (per cent)*
Œstrone	2	66
Œstrone-glycine	5	85
Œstrone-glycine	10	99
17-Ethynyl-œstradiol-17β	4	76
17-Ethynyl-œstradiol-17β	8	89
17-Hydroxycorticosterone-21-sodium-succinate	200	31
Testosterone-17-glucoside	200	76
2,6-Dimethyl-phenol	200	0
3,4-Dimethyl-phenol	200	17

* Calculated on the basis of the turbidity readings at the 20-min. mark for the uninhibited and inhibited reactions (see Fig. 2).

who recently reported that they were able to produce specific antibodies to a series of steroid residues coupled to proteins.

This work was supported by grants from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, and the Department of Health and Welfare, Canada, made to the Division of Immunochemistry and Allergy, McGill University Clinic, Royal Victoria Hospital, Montreal, Canada. We thank Drs. Eleanor McGarry and Bram Rose for their interest. Thanks are due also to Dr. Gordon Grant of Ayerst, McKenna and Harrison, Montreal, for a generous donation of œstrone, and to Dr. Walter Murphy of Ciba, Montreal, who kindly supplied samples of the testosterone and hydroxycorticosterone derivatives.

L. Goodfriend
A. H. Sehon

Departments of Biochemistry,
Chemistry and Experimental Medicine,
McGill University,
Montreal.

[1] Goodfriend, L., and Sehon, A. H., *Canad. J. Biochem. and Physiol.*, **36**, 1177 (1958).
[2] Coulson, E. J., *J. Allergy*, **24**, 458 (1953).
[3] Pressman, D., Campbell, D. H., and Pauling, L., *J. Immunol.*, **44**, 101 (1942).
[4] Gordon, J., Rose, B., and Sehon, A. H., *J. Exp. Med.*, **108**, 37 (1958).
[5] Boyden, A., Bolten, E. T., and Gemeroy, D. G., *J. Immunol.*, **57**, 211 (1947).
[6] Beiser, S. M., Erlanger, B. F., Agate, jun., F. J., and Lieberman, S., *Science*, **129**, 564 (1959).

Lack of Correlation between Synthesis of Nucleic Acids and Proteins in Denervated Muscle

After direct electrical stimulation of a muscle, non-collagen protein decreases and is later re-synthesized[1]. The rate of resynthesis is considerable, 1·9 mgm. of non-collagen protein/gm. of muscle tissue being resynthesized during 1 hr. The course of this accentuation of proteosynthesis was followed in the normal and denervated muscle. Fig. 1 shows that the decrease of proteins after stimulation of the muscle is the same in normal and denervated muscle and is accompanied by a decrease of ribonucleic acid, amounting to 15 per cent in the normal and 50 per cent in the denervated muscle. Such a decrease of ribonucleic acid and also an increase in proteolysis has been described after stimulation of nerve and brain tissue[2].

M. tib. anticus in rats was directly stimulated at a frequency of 300/min. for 6 min. Immediately, 2, 4 and 8 hr. after stimulation the muscles of the stimulated and control side were perfused with Tyrode solution. Following perfusion, both muscles were excised and the absolute amount of non-collagen proteins was determined (biuret method). The amount of ribonucleic acid was determined (Schneider's method) in another series of animals. The results are expressed in Fig. 1 as differences between the stimulated and control side. The first point of the curve corresponds to the difference between the muscle of the right and left side in control animals. Zero on the abscissæ represents the values immediately after stimulation. Proteins are expressed as μgm. non-collagen nitrogen/muscle. Ribonucleic acid values are expressed as μgm. ribonucleic acid phosphorus/100 mgm. protein nitrogen.

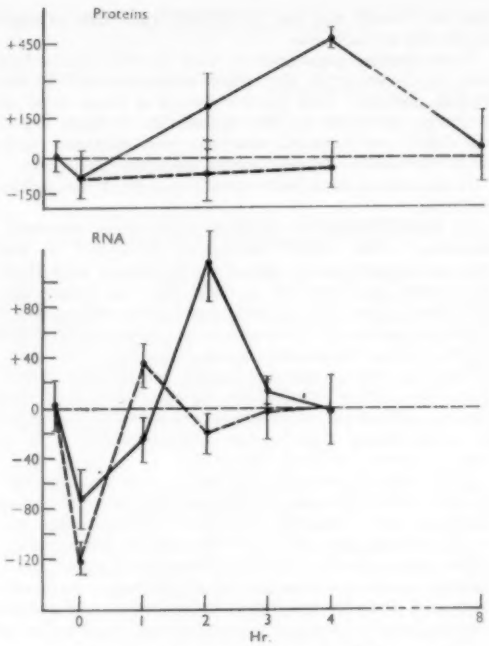


Fig. 1. Changes of non-collagen proteins and ribonucleic acid after stimulation of the normal (full line) and denervated (interrupted line) muscle

Fig. 1 shows that resynthesis of ribonucleic acid and proteins occurs after their breakdown in the normal muscle. However, the content of proteins and nucleic acids not only reaches the initial values but also their synthesis continues, the content of the proteins being 5 per cent above the initial values 4 hr. after stimulation, the content of ribonucleic acid being 22 per cent above the initial values 2 hr. after stimulation.

During the further course of the recovery phase following stimulation of the muscle, the content of proteins and ribonucleic acid returns to initial levels. The 'overshoot' reactions are apparently of general biological significance, since they have also been found during the process of resynthesis of glycogen[3] and during the restitution of the composition of ions in the muscle[4] following stimulation.

These experiments also show that the recovery of ribonucleic acid following stimulation of the muscle precedes the resynthesis of proteins. Such a time correlation between the synthesis of ribonucleic acid and proteins has also been found in, for example, regenerating liver[5] and after the administration of growth-hormone[6]. After stimulation of the denervated muscle, resynthesis of ribonucleic acid, but not of the proteins, occurs.

There is therefore a certain lack of correlation between the resynthesis of proteins and ribonucleic acid in denervated muscle. The same lack of correlation was also observed during re-alimentation following starvation, which lowers both proteins and ribonucleic acid. Table 1 shows that the ribonucleic acid content is increased by 36·5 per cent within 12 hr. after re-alimentation and that resynthesis continues so that 60 hr. later ribonucleic acid increases by 80 per cent. Compared with ribonucleic acid, proteosynthesis was found to be delayed.

Table 1. Changes in the Net Amount of Non-collagen Proteins and Ribonucleic Acid in M. tib. anticus of Rats following a Hunger Period of 10 days and after Re-alimentation

Type of experiment	Proteins (μgm. non-collagen protein N/muscle)		Ribonucleic acid (μgm. ribonucleic acid P/muscle)	
	Right side	Left side	Right side	Left side
Control animals (6 rats)	9·2±0·4	9·0 ±0·5	41·6±0·6	41·0±0·4
	$P > 0·1$		$P > 0·1$	
	Before re-alimentation	After re-alimentation	Before re-alimentation	After re-alimentation
10 days hunger and 12 hr. re-alimentation (9 rats)	5·8±0·3	5·9 ±0·2	22·1±1·9	30·2±1·5
	$P > 0·1$		$P < 0·01$	
10 days hunger and 72 hr. re-alimentation (8 rats)	6·6±0·3	8·0 ± 0·2	20·6±0·7	37·0±1·4
	$P < 0·02$		$P < 0·001$	
10 days hunger and 72 hr. re-alimentation after denervation (16 rats)	6·7±0·2	6·3 ±0·3	22·3±1·4	29·4±1·0
	$P > 0·1$		$P > 0·001$	

In all animals the muscle of one side was removed at the end of the hunger period; the muscle of the contra-lateral extremity was removed 12 and 72 hr. after re-alimentation. Denervation was performed immediately before re-alimentation.

A significant increase in non-collagen protein was found only 72 hr. after re-alimentation. If the muscle is denervated immediately before re-alimentation, there is only an increase of ribonucleic acid but no increase of non-collagen protein after 72 hr. Similar results are thus obtained in both types of experiments.

The disturbance of proteosynthesis in denervated muscle could be due to the dissociation of the synthesis of nucleic acids and proteins.

R. Žák
E. Gutmann

Institute of Physiology,
Czechoslovak Academy of Sciences,
Praha 6, Na cvičišti 2.

[1] Žák, R., Gutmann, E., and Vrbová, G., *Experientia*, **8**, 80 (1957); *Physiol. bohemoslov.*, **6**, 309 (1957).

[2] (*a*) Causey, G., and Stramann, C. J., *Biochem. J.*, **64**, 29 (1956). (*b*) Ungar, G., Aschheim, E., Psychoyos, S., and Romano, D. V., *J. Gen. Physiol.*, **40**, 635 (1957).

[3] Gutmann, E., Vodička, Z., and Vrbová, G., *Physiol. bohemoslov.*, **3**, 182 (1954).

[4] Drahota, Z., Symposium on Nervous Regulation of Metabolism and Active Transport of Ions, Prague (1958).

[5] Eliasson, N. A., Hammarsten, E., Reichard, P., Aguist, S., Thorell, A. B., and Ehrensvärd, G., *Acta Scand. Chim.*, **5**, 431 (1951).

[6] Cater, D. B., Holmes, B. E., and Mee, L. K., *Biochem. J.*, **66**, 482 (1956).

Some Interrelated Aspects of Proline and Hydroxyproline Metabolism

Proline is known to be the sole source of hydroxyproline residues in collagen[1] and is converted to hydroxyproline only after its incorporation into a polypeptide[2,3]. If proline labelled with carbon-14 is employed as a specific precursor of hydroxyproline residues of collagen, the isotope concentration in urinary excretion products derived from such residues should reflect the rate of collagen catabolism. Pyrrole-2-carboxylic acid, an important product of hydroxyproline catabolism[4], as well as 'free' hydroxyproline and 'bound' hydroxyproline, was studied in these experiments. The present communication deals with the metabolic interrelationships between these urinary excretion products. For purposes of comparison the metabolic behaviour of proline and hydroxyproline residues of skin collagen is also considered.

The pertinent results are shown in Table 1. Some points are noteworthy: (1) The rate of turn-over of 'free' hydroxyproline is identical with that of pyrrole-2-carboxylic acid, thus extending the findings of Radhakrishnan and Meister[4]. (2) The rate showing the decrease of carbon-14 activity with time indicates that in the pools of 'free' hydroxyproline and pyrrole-2-carboxylic acid two metabolically distinct components are present which have a rapid and a slow turn-over rate, respectively. (3) During the days immediately following the injection of proline-U-^{14}C, the carbon-14-activity of the 'total' hydroxyproline fraction in urine is considerably lower than that of the 'free' hydroxyproline fraction. Thereafter the turn-over rate of the 'total' hydroxyproline fraction resembles that of the 'free' hydroxyproline and pyrrole-2-carboxylic acid fractions characterized by a slow turn-over rate. (4) The hydroxyproline residues in skin collagen are labelled rapidly. One day after injection of proline-U-^{14}C about 70 per cent of all proline residues destined to be hydroxylated have been converted to hydroxyproline. These findings are in agreement with those of Stetten and Schoenheimer[1] and are similar to those of Sinex and Van Slyke[5], who studied the conversion of lysine to hydroxylysine in collagen.

The existence of two metabolically distinct components in the 'free' hydroxyproline and pyrrole-2-carboxylic acid pools may be explained in at least two ways: (1) Two different metabolic pools of collagen may exist, one representing a labile fraction which is catabolized readily to pyrrole-2-carboxylic acid or 'free' hydroxyproline and the other constituting the source of 'bound' hydroxyproline. It is conceivable that the component having a rapid turn-over represents a transitory state preceding the stabile state of the collagen molecule. On the basis of the results presented, it might be speculated that proline residues are hydroxylated most rapidly when the polypeptide is in the labile, transitory state. (2) Because of its fibrous structure, collagen may not behave in accordance with the classical concepts of metabolic turn-over. Instead of the generally accepted mechanism for *de novo* synthesis of a macromolecule, the formation of new peptide bonds might be limited to the terminal sequences of amino-acid residues of the collagen fibres. This postulate is compatible with the experimentally observed non-uniform labelling of collagen by glycine-2-^{14}C[6] and phenylalanine-3-^{14}C[7]. It might, therefore, be postulated that the probability of metabolic activation of an amino-acid residue of collagen is dependent upon the distance at which the residue is located with respect to the metabolically active end of the fibre.

Table 1. Specific Activities* of Urinary Excretion Products of Hydroxyproline Residues as well as of Proline and Hydroxyproline isolated from Skin Collagen

	Days after injection of proline-U-^{14}C			
	1	2	3	6
Pyrrole-2-carboxylic acid	126	32	7·5	2·8
'Free' hydroxyproline	110	25	6·8	3·0
'Total' hydroxyproline	6·3	4·7	3·7	2·0
'Bound' hydroxyproline†		2·8	3·2	1·8
Proline of skin collagen	39		34·5	24·5
Hydroxyproline of skin collagen	22		24	21
Ratio of proline to hydroxyproline	0·56		0·7	0·88

Each of eight rats weighing 130 ± 4 gm. was injected with approximately 6·2 μc. of proline-U-^{14}C (specific activity, 7 μc./μ*M*). Urine was then collected over several consecutive 24-hr. periods. The method devised for the isolation and determination of the urinary metabolites studied will be described in detail. Purification of skin collagen was carried out according to the procedures of Fitch (ref. 8).

* The specific activities are given as c.p.m./μ*M* for 1 μc. of proline injected per 100 gm. animal weight.

† The specific activities of 'bound' hydroxyproline were computed from the values of 'total' and 'free' hydroxyproline using average values for the ratio of 'free' to 'total' hydroxyproline concentration (that is, approximately 1 : 13 in the urine of normally fed rats).

The results of these and other work on the metabolic behaviour of collagen will be published in detail elsewhere.

This investigation has been supported in part by a contract from the U.S. Atomic Energy Commission (*AT*-(30–1)-1286) and a research grant from the U.S. Public Health Service (*C*-4687), the Cancer Institute, National Institutes of Health.

Georg B. Gerber*
Gisela Gerber
Kurt I. Altman

Division of Experimental Radiology,
University of Rochester School of Medicine,
Rochester, New York.

* Max Kade Fellow.

[1] Stetten, M. R., and Schoenheimer, R. J., *J. Biol. Chem.*, **153**, 113 (1944).
[2] Gould, B. S., and Woessner, J. F., *J. Biol. Chem.*, **226**, 289 (1957).
[3] Gould, B. S., *J. Biol. Chem.*, **232**, 637 (1958).
[4] Radhakrishnan, A. A., and Meister, A., *J. Biol. Chem.*, **226**, 559. (1957).
[5] Sinex, F. M., and Van Slyke, D. D., *J. Biol. Chem.*, **232**, 797 (1958).
[6] Gehrmann, G., Lauenstein, K., and Altman, K. I., *Arch. Biochem Biophys.*, **62**, 509 (1956).
[7] Gehrmann, G., Lauenstein, K., and Altman, K. I., *Arch. Biochem. Biophys.* (in the press).
[8] Fitch, S. M., *Nature*, **176**, 173 (1955).

Copper(II) Complexes of Histamine and 3-Methyl Histamine

In previous communications from this laboratory[1–3] evidence has been presented that acid dissociations occur from the 1 : 1 cupric complexes of histidine and histamine. In the presence of equimolar cupric chloride, one equivalent of alkali additional to that needed by either of the ligands alone is required to titrate all the acid produced. When the ligand/copper(II) ratio is 2 : 1 the amount of alkali required is the same as in the absence of the metal. From these facts it was concluded[3] that a co-ordinated water molecule and not the hydrogen attached to the unco-ordinated imidazole nitrogen was the source of the acid ionization from the 1 : 1 complexes. As a further test of this idea, we have investigated the copper(II) complexes of 3-methyl histamine.

Titration curves of 3-methyl histamine dihydrochloride in the absence and presence of cupric chloride are shown in Fig. 1. It can be seen that an acid ionization occurs from the 1 : 1 and not the 2 : 1 complex. Thus histamine and its 3-methyl derivative are similar in their co-ordinating properties with copper(II), and the interactions which occur may be represented thus :

$$Cu^{2+} + L \overset{K_1}{\rightleftharpoons} CuL^{2+} \quad (1)$$

$$CuL^{2+} \overset{K_c}{\rightleftharpoons} CuL^{+} + H^{+} \quad (2)$$

$$CuL^{2+} + L \overset{K_2}{\rightleftharpoons} CuL_2^{2+} \quad (3)$$

where

$$K_1 = \frac{[CuL^{2+}]}{[Cu^{2+}][L]}, K_c = \frac{[CuL^{+}][H^{+}]}{[CuL^{2+}]}, K_2 = \frac{[CuL_2^{2+}]}{[CuL^{2+}][L]}$$

The constants were calculated by the methods previously described[3] and the numerical values obtained did not vary significantly with *p*H (at 25° C. $pK_{a1} = 5{\cdot}80$, $pK_{a2} = 9{\cdot}90$, $\log K_1 = 9{\cdot}58$, $\log K_2 = 6{\cdot}56$, $pK_c = 7{\cdot}10$, where K_{a1} and K_{a2} are the acid dissociation constants of the ligand). These values are not very different from those reported for histamine[3].

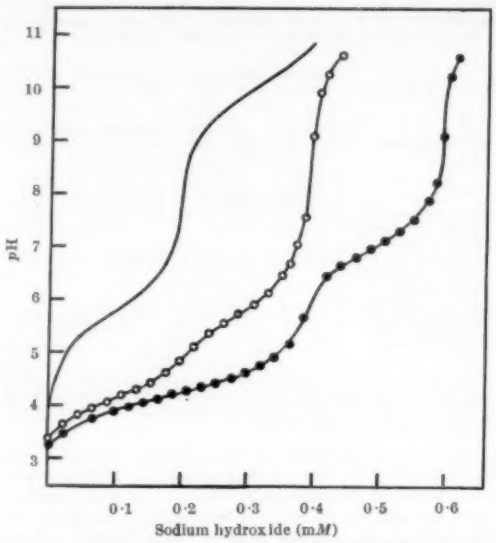


Fig. 1. Titration curves of 3-methyl histamine dihydrochloride (0·2 m*M* in 20 ml.) with sodium hydroxide (0·11 *N*): ——, no additions; —○—○—○—, plus 0·1 m*M* cupric chloride; --●--●--●--●--, plus 0·2 m*M* cupric chloride

The hydrogen attached to the unco-ordinated imidazole nitrogen cannot be the source of the acid dissociation from the histamine complex, since this hydrogen is not present in 3-methyl histamine and it is most unlikely that the two ligands differ in the nature of the complexes they form. The evidence, therefore, favours the previous suggestion that the acid dissociation (equation 2) occurs from a water molecule co-ordinated to the 1 : 1 complex, CuL^{2+}, or the equivalent process, the uptake by this complex of a hydroxyl ion.

We are indebted to Dr. R. W. Schayer, Merck Institute of Therapeutic Research, for kindly supplying the 3-methyl histamine dihydrochloride used in this investigation.

R. Leberman
B. R. Rabin

Department of Biochemistry,
University College London,
Gower St., London, W.C.1.

[1] Datta, S. P., Leberman, R., and Rabin, B. R., *Biochem. J.*, **68**, 22*P* (1958).
[2] Leberman, R., and Rabin, B. R., *Nature*, **183**, 746 (1959).
[3] Leberman, R., and Rabin, B. R., *Trans. Farad. Soc.*, **55**, 1660 (1959).

ANIMAL PHYSIOLOGY

Renal Affection resulting from Blood Trauma in Extracorporeal Circuits

Previously, we have presented evidence that as a result of the corpuscular blood trauma inherent to extracorporeal circulatory systems including the heart–lung machines, adenosine triphosphate and 5-hydroxytryptamine (serotonin) are released into plasma, the former being derived mainly from the hæmolysed red cells and the latter from the disintegrated platelets. Concurrently, the urinary excretion of 5-hydroxytryptamine was increased, while, rather unexpectedly, there was a reduction in the

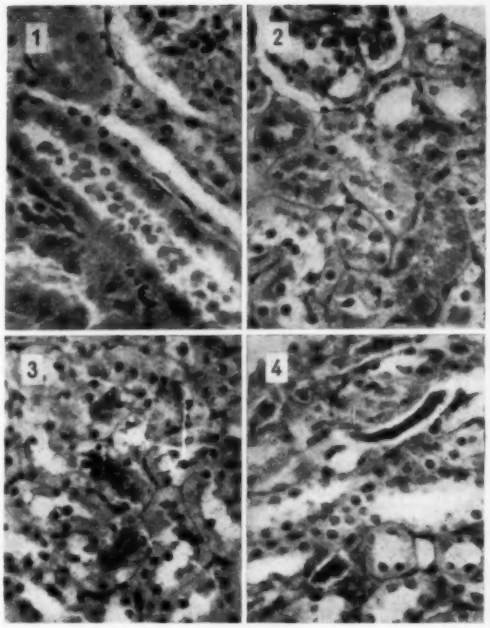
excretion of 5-hydroxyindoleacetic acid, the main excretory product of hydroxytryptamine[1]. Since intravasal hæmolysis caused in any way may evoke an acute tubular ('lower nephron') nephrosis[2,3] and since, on the other hand, 5-hydroxyindoleacetic acid has been reported to be excreted also by active tubular secretion[4], we have studied the renal changes in a part of the animals, in which the above blood and urinary changes were found.

The animal material studied comprised four rabbits and three dogs. The experimental procedure was described in our previous report[1]. In brief, the animals were pretreated with pethidine hydrochloride, anæsthetized with pentobarbitone sodium and heparinized, following which an arteriovenous (femoral artery-superficial jugular vein) shunt, consisting of a specially prepared polythene tube, was created. In the period of this extracorporeal circulation, maintained for 90 min., the blood and urinary changes were observed. In general, the animals were killed at the termination of extracorporeal circulation, except one dog, in which the experiment was repeated one week later. At autopsy, both the kidneys were excised and fixed in neutral 10 per cent formalin. The embedded material was serially sectioned and stained with hæmalum–eosin and hæmalum–periodic acid Schiff-orange *G*. Frozen sections were stained with a mixture of sudan III and sudan IV for fats and with benzidine for hæmoglobin.

Pathological renal changes were found in both rabbits and dogs. In rabbits the changes generally consisted of some glomerular congestion associated with a leakage of eosinophilic, periodic acid–Schiff-positive material into the glomerular space (Fig. 1). The proximal convoluted tubuli showed a more or less intense intracellular deposition of orange *G*-positive material (Fig. 2), which also gave a positive benzidine-reaction. In addition, eosinophilic, orange *G*-positive material, in the form of granular bodies, droplets (Fig. 1) or plugs (Fig. 3), were seen especially in the lumina of the proximal convoluted tubuli. In the lower portions of tubuli there were eosinophilic, intensely periodic acid–Schiff-positive casts (Fig. 4). No definite destructive changes were observed in the tubular epithelium ; but in the intertubular vascular bed of the medulla there was a patchy type of congestion with occasional thrombosis. Fatty changes were consistently lacking. In dogs, changes of the same general type were encountered ; but they were less pronounced and the patchy vascular changes were absent. In the three rabbits and three dogs studied for comparison none of the changes described were seen.

Accordingly, the pathological renal changes observed largely correspond to those typical of the so-called 'lower nephron nephrosis'[2,3]. The lack of any definite destructive tubular changes may well be related to the short reaction time in the present experiments. The observation that the renal changes were less pronounced in dogs agrees with our finding, previously reported[1], that the corpuscular and humoral indices of blood trauma also were less pronounced in dogs. This also demonstrates that the renal changes under consideration are pathogenetically related to the blood trauma observed in our animals[1].

The glomerular and tubular structures were not affected throughout. On the other hand, the dog, in which the experiment was repeated one week later, did not show any pathological renal changes suggestive of irreversibility. Thus, the renal changes described



Figs. 1–4

can scarcely be regarded as being serious, especially *quo ad vitam*. Yet the present finding may be of significance at least in two respects. First, the unexpected reduction in the excretion of 5-hydroxyindoleacetic acid, as observed in our animals[1], may be related to some injury to the proximal convoluted tubuli, to which monoamine oxidase, essential for the conversion of 5-hydroxytryptamine into 5-hydroxyindoleacetic acid, is localized in the kidney[5] and in which the active secretion of 5-hydroxyindoleacetic acid presumably takes place[4]. In this respect, however, definite conclusions are not warranted, because the plasma-levels of 5-hydroxyindoleacetic acid were not observed in our animals and because detailed information on the mode of excretion of 5-hydroxytryptamine is not available for the present. Secondly, as pointed out previously[1], the corpuscular blood trauma in the present experiments was of the same general degree as that in the current heart–lung machines. A comparable degree of renal damage may therefore occur following the use of the current heart–lung machines for open cardiac surgery. Such renal damage may even be aggravated, should severe hypotension, as has been reported[6], ensue as a post-perfusion complication. It may be recalled that Daly and associates[7] found more severe renal changes, with occasional tubular necroses, in dogs perfused for about 3 hr. by means of a pump-oxygenator. They further found congestion, hæmorrhages and necrobiotic changes in other vital organs and were inclined to ascribe these changes as well as the allied signs of peripheral circulatory shock to 'vasotonins' appearing in the blood during perfusion. Our findings, previously discussed[1], suggest that adenosine triphosphate and 5-hydroxytryptamine may be the 'vasotonins' involved.

Finally, Brull and Louis-Bar[8] have recently established the toxicity of artificially circulated, heparinized blood to the kidney and speculated that

5-hydroxytryptamine released from disintegrated platelets might be the causative agent. In their experience, furthermore, this toxic action could not be prevented by siliconizing the foreign channels.

The present studies have been supported by the Sigrid Jusélius Foundation and Medica, Ltd., Helsinki, and by the Upjohn Company, Kalamazoo, Mich.

H. S. S. Sarajas

Institute of Physiology,
University of Helsinki,
and
Wihuri Research Institute.

L. Saure

Zoological Institute,
University of Helsinki.

[1] Sarajas, H. S. S., Kristoffersson, R., and Frick, M. H., *Nature*, **184**, 1127 (1959); *Amer. J. Physiol.*, **197**, 1195 (1959).
[2] Burch, G. E., and Ray, C. T., *Ann. Int. Med.*, **31**, 750 (1959).
[3] Ogilvie, R. F., "Pathological Histology" (E. and S. Livingstone, Ltd., Edinburgh, 1951).
[4] Despopoulos, A., and Weissbach, H., *Amer. J. Physiol.*, **189**, 548 (1957).
[5] Blaschko, H., and Hellmann, K., *J. Physiol.*, **122**, 419 (1953).
[6] Schmutzer, K. J., Marable, S. A., Ruschke, E., Maloney, J. V., and Longmire, W. P., *Langenbecks Arch. klin. Chir.*, **290**, 54 (1958).
[7] Daly, I. De Burgh, Eggleton, P., Hebb, Catherine, Linzell, J. L., and Trowell, O. A., *Quart. J. Exp. Physiol.*, **39**, 29 (1954).
[8] Brull, L., and Louis-Bar, D., *Arch. Internat. Physiol. et Biochem.*, **65**, 470 (1957).

Loss of Heat by Evaporation in Young Lambs

Animals exposed to temperatures approaching or exceeding that of the body can lose little or no heat by radiation, convection and conduction; they must rely partly or wholly on evaporative cooling from the skin or respiratory tract to maintain homeothermy.

Although sheep have been shown to have a high heat tolerance[1,2], no direct measurements of loss of water from the skin and from the respiratory tract have been reported for this animal. Such measurements have now been made in young lambs subjected to a wide range of environmental temperatures, and the relative importance of the two routes of evaporative heat loss has been assessed.

Four Merino lambs were examined during the first fifteen days of life; they were fasted for 4–12 hr. before each experiment, which lasted 1–1½ hr. The experiments were made in a closed-circuit respiration apparatus (indirect calorimeter) previously outlined[3], but here modified so that respiratory and cutaneous water losses could be determined separately. The lamb, in the chamber of the apparatus, respired into an air-tight polythene mask which covered the head and through which air was circulated. Oxygen was metered into the mask from a spirometer, and expired water vapour and carbon dioxide were determined by weighing. Cutaneous water-loss from the body and limbs was measured by weighing the water absorbed from the air circulating within the chamber. Water vapour pressures at the mask outlet and in the chamber were usually maintained within 3 mm. mercury of each other; they ranged from about 3 mm. mercury at 8° C. to 20 mm. mercury at 45° C. Turbulent air flow over the lamb was at the rate of about 30 cm./sec. Measurement of loss of water, etc., was usually not begun until ¾–1 hr. after the lamb had first been subjected to the experimental conditions.

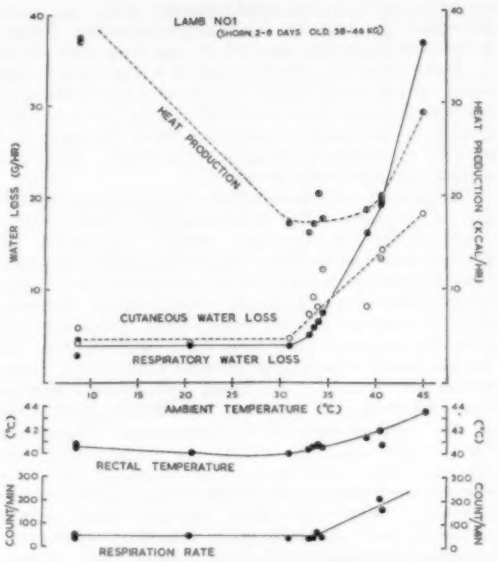


Fig. 1. Heat production, loss of water by evaporation, rectal temperature and respiration-rates in a shorn lamb at various ambient temperatures

The results from lamb No. 1 on which most observations were made are shown in Fig. 1. This lamb had been shorn closely to eliminate errors due to release or absorption of water by the fleece during the experiments. At ambient temperatures below 30° C., losses of water from the skin and from the respiratory tract were relatively constant, the former slightly exceeding the latter. Above 30° C., loss of water from both sources increased with increasing temperature, the rate of increase of the respiratory loss being the greater. Heat production and rectal temperature were minimal at 30° C.; respiration-rate was constant below 30° C. and increased above 30° C. Similar qualitative results were obtained in three other lambs which were not shorn (Fig. 2).

The break in the curve relating ambient temperature to cutaneous water loss suggests that an active

Table 1. Evaporative Water Loss from Lambs with Cutaneous or Respiratory Channel Blocked. Ambient Temperature, 40–41° C.

Lamb	Channel blocked	Water loss (gm./hr.)		Heat production (kcal./hr.)	Rectal temperature (° C.) during experiment	
		Cutaneous	Respiratory		At start	After 1 hr.
No. 1 (shorn)	Neither	14·3	19·5	19·3	40·8	40·8
Age: 8 days	Cutaneous—'completely'	*	32·4	21·9	41·8	41·8
Weight: 4·7 kgm.	Respiratory—partly†	17·2	*	30·7	41·3	43·3
No. 5 (unshorn)	Neither	11·7	36·7	34·3	42·3	42·9
Age: 15 days	Cutaneous—'completely'	*	54·6	42·0	43·7	44·8
Weight: 7·3 kgm.	Respiratory—partly†	11·2	30·6	42·5	42·1	44·9

* Not determined.
† Water vapour pressure was gradually increased from 15 to 55 mm. mercury during the experiment.

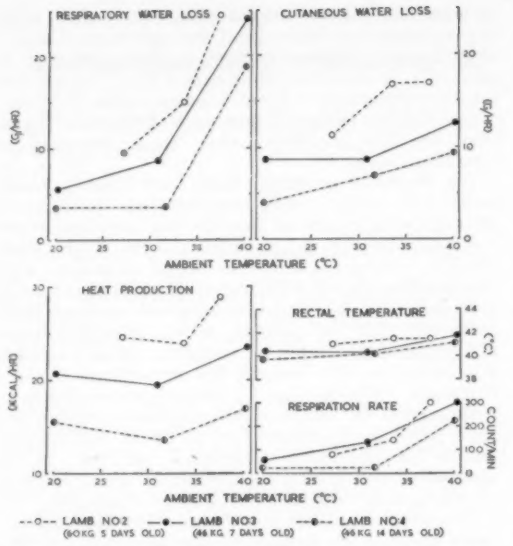


Fig. 2. Heat production, loss of water by evaporation, rectal temperature and respiration-rates in three unshorn lambs at various ambient temperatures

process had come into play above 30° C.; that is, sweating occurred.

The functional reserve of each channel of evaporative cooling was examined in two lambs at 40–41° C. by raising the humidity close to saturation in one channel, to reduce or virtually prevent evaporation, and measuring water loss from the other (Table 1).

In lamb No. 1 when loss of heat by evaporation from the skin was blocked, the loss by respiration was increased to compensate and it was able to prevent a severe rise in rectal temperature. On the other hand, when loss by respiration was much reduced, cutaneous loss was not capable of any marked response and rectal temperature rose rapidly. Although lamb No. 5 was unable to keep its rectal temperature down even when neither channel was blocked, its responses were similar to those of lamb No. 1.

These findings show that, in a hot environment, lambs lose heat by both panting and sweating, and that in the region of minimal heat production the contribution of each is about equal. However, at higher ambient temperatures, the contribution of panting becomes by far the greater, and there is a great functional reserve of evaporative cooling by this route. Sweating appears to approach its limit at that ambient temperature where heat losses by radiation, convection and conduction cease (Table 1).

Published data on adult sheep suggest that they, too, sweat[1,4,5]; but there is insufficient evidence to indicate whether the adult shows the same quantitative relation between sweating and panting as the young. However, results with lambs suggest that in the sheep the relative importance of sweating and panting differs from that in the few other species for which data are available. In the dog[6], sweating is of no consequence, while in the camel[7] and man it is virtually the sole means of evaporative cooling. Cattle, too, appear to rely mainly on sweating[8], but loss of water by respiration shows some increase in heat stress. In sheep, however, panting is apparently the more important mechanism of heat elimination, but sweating can contribute substantially to evaporative cooling.

G. Alexander
A. H. Brook

Commonwealth Scientific and Industrial Research Organization,
Division of Animal Health and Production,
Sheep Biology Laboratory,
P.O. Box 144, Parramatta,
New South Wales.

[1] Lee, D. H. K., and Robinson, K. W., *Proc. Roy. Soc. Qd.*, **53**, 189 (1941).
[2] Macfarlane, W. V., Morris, R. J. H., and Howard, B., *Aust. J. Agric. Res.*, **9**, 217 (1958).
[3] Alexander, G., *Proc. Aust. Soc. Anim. Prod.*, **2**, 10 (1958).
[4] Riek, R. F., Hardy, M. H., Lee, D. H. K., and Carter, H. B., *Aust. J. Agric. Res.*, **1**, 217 (1950).
[5] Knapp, B. J., and Robinson, K. W., *Aust. J. Agric. Res.*, **5**, 568 (1954).
[6] Hammel, H. T., Wyndham, C. H., and Hardy, J. D., *Amer. J. Physiol.*, **194**, 99 (1958).
[7] Schmidt-Nielsen, K., Schmidt-Nielsen, B., Jarnum, S. A., and Houpt, T. R., *Amer. J. Physiol.*, **188**, 103 (1957).
[8] Taneja, G. C., *J. Agric. Sci.*, **50**, 73 (1958).

Effect of β-Hydroxybutyrate Injections on the Pancreatic Activity of Guinea Pigs and Rats

A product of the intermediary metabolism of fats, namely, β-hydroxybutyric acid, appears in excess during disturbed metabolic processes, especially in diabetic organisms. The accumulation of this intermediary product causes different pathological conditions (acidosis, etc.) in the animal. M. C. Nath and H. D. Brahmachari[1] and H. D. Brahmachari and Mahendra Kumar[2] conclude that chronic administration of β-hydroxybutyrate to guinea pigs and rabbits causes, after a transitory hypoglycæmia, a permanent hyperglycæmia and a lowered glucose tolerance, hypo-insulinism due to atrophy through over-stimulation of the *B*-cells of the pancreas.

Similar observations have been made in our laboratory[3], but after chronic administration of adrenocorticotrophic hormone. We concluded that the parenteral administration of β-hydroxybutyrate produces a mobilization of the adrenocorticotrophin from the hypophysis and, as a consequence, the symptoms observed by Brahmachari *et al.*[2] may be secondary processes due to excess production of corticosteroids as a consequence of the mobilization of adrenocorticotrophic hormone.

We have therefore extended the investigation. Experiments have been carried out on guinea pigs and on white rats of both sexes, divided into four experimental lots, each composed of 5 animals: (1) Normal rats and guinea pigs; (2) hypophysectomized rats; (3) normal rats and guinea pigs treated with β-hydroxybutyrate, 50–100 mgm. per animal; (4) hypophysectomized rats treated with the same dose of β-hydroxybutyrate.

The following determinations were made on each animal: (*a*) extent of glycæmia (photocolorimetrical method, Somogyi–Nelson[4,5]); (*b*) quantitative estimation of plasma-insulin (Vallance-Owen *et al.*[6]); (*c*) estimation of adrenocorticotrophic hormone in blood plasma (Sayers *et al.*[7], modified by us[8]).

The results given in Table 1 demonstrate clearly that during the administration of β-hydroxybutyrate (sodium salt) the glycæmia, after a temporary lowering, rises to 210 mgm. per cent. At the same time the quantity of plasma insulin—after a considerable

Table 1. Effect of the Chronic Treatment with Sodium β-Hydroxybutyrate on Normal Animals

No.	Days of injections	Glycæmia (mgm. per cent)	Insulin in plasma (microunits/ml.)	Adrenocorticotrophic hormone in plasma (milliunits/100 ml.)
1	0 (control)	98	92 ± 6	0·5 ± 0·1
2	7	69	310 ± 18	3·5 ± 3·1
3	14	71	360 ± 11	2·1 ± 2·3
4	28	110	135 ± 8	3·3 ± 1·1
5	35	180	60 ± 18	9·3 ± 3·1
6	42	210	30 ± 11	5·8 ± 5·1

increase—decreases to less than the normal values, whereas the level of adrenocorticotrophic hormone from the blood rises to about 10 times that of the initial values, that is to say, the normal values.

We observed in rats treated for 14, 28 and 42 days with this product a destruction of pancreatic *B*-cells, a destruction similar to that observed in rats treated chronically with adrenocorticotrophic hormone, as described by us[3].

These results confirm Brahmachari's observations concerning the diabetogenic effect of the β-hydroxybutyrate.

In order to analyse the mechanism of the action of this substance, we subjected some rats to hypophysectomy, afterwards treating them chronically with this intermediary product. We supposed that if the β-hydroxybutyrate would attack the *B*-cells directly, then even in the absence of the pituitary gland (absence of adrenocorticotrophic hormone) the diabetogenic effect would still take place. But if the pancreas remains intact, that is to say, if the symptoms described above do not appear, then we can assert that this diabetogenic effect of β-hydroxybutyrate is not a primary one, but is mediated by the pituitary gland.

Our results (Table 2) with the hypophysectomized rats after a chronic treatment with sodium β-hydroxybutyrate show an oscillation of the plasma insulin values, but within normal limits. Glycæmia also shows an increase but of little importance. Histologically no destruction of the *B*-cells was observed even after a longer treatment.

We then treated a series of hypophysectomized rats with sodium β-hydroxybutyrate + adrenocorticotrophic hormone (0·5 μgm./100 gm.). In these animals the plasma insulin value decreased, some hyperglycæmia occurred, and the histological aspect of the *B*-cells showed signs of partial atrophy in general. It must be emphasized that though the β-hydroxybutyrate + adrenocorticotrophic hormone, in the absence of hypophysis, provoke diabetic signs, they are less important than those observed in normal animals.

Table 2. Effect of the Chronic Treatment with Sodium β-Hydroxybutyrate upon Hypophysectomized Animals

No.	Days of injections	Glycæmia (mgm. per cent)	Insulin in plasma (microunits/ml.)	Adrenocorticotrophic hormone in plasma (milliunits/100 ml.)
1	0 (control)	88	44 ± 4	0·0 ± 0·0
2	7	91	66 ± 1·3	—
3	14	90	49 ± 7	—
4	28	118	71 ± 11	—
5	35	101	31 ± 6	—
6	42	87	91 ± 8	—

Table 3. Effect of the Sodium β-Hydroxybutyrate + Adrenocorticotrophic Hormone Treatment on Hypophysectomized Animals

No.	Days of injections	Glycæmia (mgm. per cent)	Insulin in plasma (microunits/ml.)	Adrenocorticotrophic hormone in plasma (milliunits/100 ml.)
1	0 (control)	78	60 ± 10	0·0 ± 0·0
2	7	130	120 ± 21	—
3	14	127	150 ± 11	—
4	28	140	99 ± 0	—
5	35	121	60 ± 8	—
6	42	154	40 ± 1	—

We therefore conclude that: (1) Chronic treatment with sodium β-hydroxybutyrate produces a diabetic state in normal animals, but the effect is not a direct one; it takes place via the hypophysis. (2) The consequences of the chronic treatment with this product are very similar to those obtained by us with a longer treatment with adrenocorticotrophic hormone. (3) The diabetic effect upon the hypophysectomized animals treated with sodium β-hydroxybutyrate has not the importance initially supposed. The sodium β-hydroxybutyrate leads to mobilization of adrenocorticotrophic hormone and there may be hypersecretion of other diabetogenic factors (somatotrophic-hormone); and thus in the absence of the pituitary gland, even in the presence of exogenous adrenocorticotrophic hormone, the diabetic signs are less significant. (4) The diabetogenic effect of the sodium β-hydroxybutyrate as well as of the adrenocorticotrophic hormone are diminished in the absence of the pituitary gland.

A. Schwartz
J. Manta
J. Madar
Z. Kis

Institute of Animal Physiology,
University Babes-Bolyai,
Cluj. Republica Populara Română.

[1] Nath, M. C., and Brahmachari, H. D., *Nature*, **161**, 18 (1948).
[2] Brahmachari, H. D., and Mahendra Kumar, *Nature*, **183**, 51 (1959).
[3] Schwartz, A., Madar, J., and Kis, Z., *Studii si Cercet. de Medicina Academia RPR. Cluj.*, No. 3–4, 291 (1957).
[4] Somogyi, M., *J. Biol. Chem.*, **160**, 61 (1945).
[5] Nelson, N., *J. Biol. Chem.*, **153**, 375 (1944).
[6] Vallance-Owen, J., and Hurlock, B., *Lancet*, I, 68 (1954).
[7] Sayers, M. A., and Sayers, G., *Endocrinol.*, **42**, 379 (1948).
[8] Schwartz, A., Madar, J., and Kis, Z., *Studii si Cercet. de Biologie Academia RPR. Cluj.*, No. 1–2, 46 (1958).

Vasodepressor Effect of Adenosine Phosphates and 5′-Adenylmethylene-diphosphonic Acid, an Analogue of Adenosine Triphosphate

Members of the adenosine, adenosine monophosphate, adenosine diphosphate and adenosine triphosphate series produce a marked vasodepressor effect in the cat anæsthetized with nembutal. In this series, as the number of phosphate groups in the 5′ position is increased, the vasodepressor effect is also increased[1].

Two general hypotheses as to the order of potency in the members of this series may be considered. In the first, cleavage of the polyphosphate side-chain is postulated with or without transfer of phosphate to a substrate or active site. The high activity of adenosine triphosphate in dilating the coronary arteries in the dog led Winbury *et al.*[2] to suggest that "bond energy may serve to trigger some biochemical process leading to greater dilatation".

Alternative explanations for the mechanism of the action of members of this series, which would not involve 'high-energy' bond cleavage, might be found in the complexing or binding capacity of these

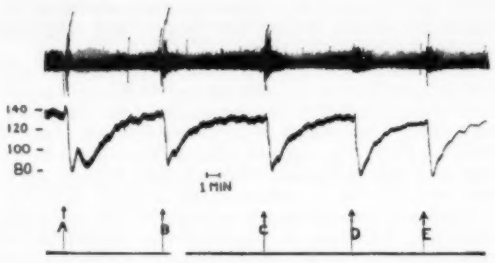
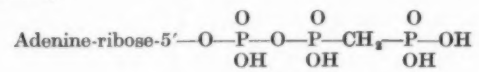


Fig. 1. Respiration (upper) and arterial blood pressure in mm. mercury (lower) tracings from a 2 kgm. cat (male) anæsthetized with nembutal. The letters indicate intravenous injection of the following: (*A*) 1 μmole analogue; (*B*) 4 μmoles adenosine monophosphate; (*C*) 4 μmoles adenosine monophosphate; (*D*) 2 μmoles adenosine triphosphate; (*E*) 2 μmoles adenosine triphosphate

compounds. Martell and Schwarzenbach[3] have shown that the association constants of the members of this series, with magnesium and calcium complexes, increase with the number of phosphate groups. Thus the vasodepressor activity appears to parallel the change in association constants of these compounds.

The present preliminary report describes the results obtained in comparing the vasodepressor effect of adenosine monophosphate and adenosine triphosphate with 5′-adenylmethylenediphosphonic acid, a phosphonic acid analogue of adenosine triphosphate (this compound has been prepared and characterized by us; details will be given elsewhere).



5′-adenylmethylenediphosphonic acid

Experiments were performed on thirty-one cats anæsthetized with nembutal. The carotid blood pressure was recorded with a mercury manometer on a kymograph. Adenosine mono- and tri-phosphate and the analogue were administered intravenously in 0·9 per cent sodium chloride.

Fig. 1 illustrates the results obtained in a cat anæsthetized with nembutal, in which the magnitude of the vasodepressor response is about the same for each of the doses used. The relative potency of the analogue varied from 1·2 to 4·2 times that of adenosine triphosphate in these experiments, with a mean value of 2·3 for 31 such comparisons.

Adenosine monophosphate was found to have a relative potency of 0·22–0·50 that of the triphosphate, with a mean value of 0·33.

The methylene group in place of the pyrophosphate oxygen of adenosine triphosphate results in an increase in vasodepressor activity. This indicates that the terminal pyrophosphate group of the triphosphate is neither necessary for activity nor for enhancement of activity. It is well established that the phosphonic acid (C–P bond) is markedly resistant to chemical cleavage[3,4]. Additional evidence of this resistance to cleavage was seen when the analogue was substituted for adenosine triphosphate in the yeast hexokinase reaction, which reaction has been proved to take place with the cleavage of the adenosine triphosphate terminal pyrophosphate bond[5]. The analogue failed to serve as a substitute for adenosine triphosphate in this reaction. Since the similarity of the pharmacological effects of adenosine triphosphate and its phosphonic acid analogue are striking, this also suggests that cleavage of the terminal pyrophosphate bond is not essential for the production of the vasodepressor effect by adenosine triphosphate. The possibility that the analogue may owe its activity to cleavage of the second pyrophosphate bond, giving rise to adenosine monophosphate and methylenediphosphonic acid, which in turn produces the observed response, seems unlikely since these compounds given together show much less activity than the analogue.

Considering the results reported here, the mechanism of action of the series of compounds may be due to binding to the receptor site without a cleavage of the chain. The activity of the analogue could be accounted for in this manner; that is, binding to the receptor site without cleavage of the chain. The increase in activity of the analogue in comparison with the other compounds may result from a greater affinity for this receptor site.

James W. Flesher
Y. T. Oester

Department of Pharmacology and Therapeutics,
Stritch School of Medicine of Loyola University,
Chicago 12, Illinois.

T. C. Myers

Department of Biochemistry,
University of Illinois College of Medicine,
Chicago, Illinois.

[1] Green, H. M., and Stoner, H. B., "Biological Actions of the Adenine Nucleotides" (H. K. Lewis and Co., London, 1950).
[2] Winbury, M. M., Paplerski, D. H., Henmer, M. L., and Hambourger, W. E., *J. Pharm. Exp. Therap.*, **109**, 225 (1953).
[3] Martell, E. E., and Schwarzenbach, G., *Helv. Chim. Acta*, **39**, 653 (1956).
[4] Kosalopoff, G. M., "Organophosphorus Compounds" (Wiley and Co., New York, 1950).
[5] Colowick, S. P., and Kalckar, H. M., *J. Biol. Chem.*, **148**, 117 (1943).

Effect of Different Types of Milk on Intestinal Motility

In conditions of infantile diarrhœa restriction of milk from the diet causes rapid improvement of the condition: if the milk diet is resumed too early, the symptoms rapidly appear again. This shows that, most probably, there is a relation between the milk and the symptoms of infantile diarrhœa. It may be that in the milk there are factors which stimulate the motility of the small intestine and thus cause its rapid evacuation.

It was found by Becovic and Moglajile[1] that mare's milk contains acetylcholine. This was shown by studying the effect of milk on the isolated frog's heart and the small intestine of the dog prepared *in situ* according to the method of Straub and Triendler. The effects of human milk and the different types of milk obtained from Egyptian dairy animals on the intestinal motility *in vitro* were therefore examined. Fresh milk, stored milk, skimmed milk and pasteurized milk were used.

The jejunum of freshly killed rabbit of about 1 kgm. body-weight was excised. Isolated pieces of jejunum of about 3 cm. in length were suspended in an organ bath of about 10 ml. volume containing oxygenated Tyrode's solution maintained at 37° C. The movements of the longitudinal muscle were recorded by a lever. The normal action and the effect of adding a few drops and 2 c.c. of different types of milk were

compared. When the milk showed an excitatory response, its effect was studied on an atropinized loop of intestine.

Fresh milk was collected from the animal by hygienic methods and was immediately examined.

Fresh cow's milk. A few drops had no effect on the intestinal motility. When 2 c.c. of the milk (at 37° C.) were added to the intestinal bath, it caused temporarily slight inhibitory effect after which the intestinal motility gradually increased. Addition of one drop of bellafolin immediately stopped the excitatory effect.

Fresh buffalo's milk. A few drops of the milk added to the intestinal loop caused immediate inhibition of the intestinal motility; this inhibitory effect disappeared very rapidly and the intestine recovered. Addition of 2 c.c. of fresh milk to the intestinal bath caused a marked inhibitory effect, and then the intestine gradually recovered.

Fresh goat's milk. Complete immediate relaxation of the intestinal loop was obtained by the addition of a few drops. A high dose of 2 c.c. produced a similar effect. Recovery gradually occurred.

Fresh human milk. Whether fresh or stored in the refrigerator, human milk had no effect on the motility of the intestinal loop.

The effect of stored milk at room temperature up to 12 hr. or at very low temperature (0° C.) up to 7 days did not cause any change in the behaviour of the intestinal loop.

Pasteurization of milk did not change its effect on the intestinal motility.

The effects of different types of whey were examined.

Cow's whey. Whether in small dose of 5 drops or a big dose of 2 c.c., cow's whey caused a preliminary inhibition followed by strong excitatory effect. One drop of bellafolin immediately stopped the excitatory effect. Cow's whey in high doses did not show the excitatory effect on the atropinized loop.

Buffalo's whey. This produced complete relaxation of the intestinal loop when added even in very small doses.

Goat's whey. The inhibitory effect which was recorded in the case of fresh, stored, and pasteurized goat's milk cannot be shown in case of the whey, but it caused a very strong excitatory effect which is not produced in the case of the atropinized loop.

The preliminary inhibitory effect observed in the case of fresh cow's milk and the maintained inhibitory effect of buffalo's milk were not shown when the milk was previously boiled at atmospheric pressure and then added to the intestinal bath after cooling to 37° C. In the case of goat's milk, boiling did not abolish the inhibition produced.

When the anti-histaminic 'Sandosten' was added to the Tyrode solution in the intestinal bath, the normal effect produced by the different milks were not changed.

The experiments show that different milks have different effects as regards the motility of the intestinal loop *in vitro*. Cow's milk, whether fresh, stored, pasteurized or boiled, had an excitatory effect. The parasympatholytic drug bellafolin abolished this excitatory response. Buffalo's and goat's milk showed a maintained inhibitory effect. As these effects could be observed when the whey was used, the factor concerned with excitation or inhibition of the intestinal loop most probably lies in the whey. Becovic and Moglajile[1] stated that mare's and cow's milk contain acetylcholine, and as the Egyptian cow's milk showed an excitatory effect abolished by bellafolin, so this finding by Becovic and Moglajile can be applied to this milk.

The inhibitory effect produced by goat's milk and buffalo's milk may be due to an inhibitory factor, the nature of which is not yet settled. As the inhibitory effect was abolished by boiling in the case of buffalo's milk, but was not affected by boiling in the case of goat's milk, this indicates that the nature of the inhibitory factor differs in the different milks.

Human milk had no effect at all on the intestinal motility. This may be due either to the absence of such excitatory or inhibitory factors, or they may be present in concentrations antagonizing each other.

The study of the effect of the different amino-acids present in milks and the nature of the excitatory and inhibitory factors is under investigation in our laboratories.

A. H. Mohamed
O. Zaki
K. Zaki
L. Fahim

Department of Physiology,
Faculty of Medicine,
Ein Shams University,
Abbassia, Cairo.

[1] Becovic, S., and Moglajile, E., *Wiener Tierarztl. Monatsschr.*, **42** (2), 68 (1955).

Hormonal Control of Secretion in the Oviduct of the Amphibia

Galli-Mainini[1], Houssay[2] and Allende[3] have shown that secretion by the glands of the oviduct in *Bufo arenarum* is controlled by a dual mechanism: a prolactin-like hormone from the anterior pituitary acts directly on the oviduct, and a progesterone-like hormone produced in the ovary during ovulation causes secretion independently of the direct pituitary action. In view of the importance from the evolutionary point of view of this demonstration of the action of a progestational hormone at the level of the Amphibia, it was thought desirable to extend the work to include the two British species, *Bufo bufo* and *Rana temporaria*.

In the first series of experiments various concentrations of progesterone ('Progestin' B.D.H.) in 0·2 ml. arachis oil were injected into the lumina of the doubly ligatured left oviducts of ovariectomized frogs and toads[3]. In all experiments using toads the anterior lobe of the pituitary had been removed at least two days previously. 48 hr. after injection the animals were killed, and, after weighing the oviduct and measuring the length enclosed by the two ligatures, the gelatinous secretion within the oviduct was removed and weighed. The results were expressed as the ratio of the weight of secretion in mgm. (after correcting for the weight of oil injected) to the length of oviduct between the ligatures in cm. This expression is termed the quotient of secretion[1,3]. The quotient is numerically smaller in the frog, because, unlike the condition found in the toad, there is no clear differentiation of the oviduct into two parts and it was necessary to use the whole length of the oviduct if the quotients from different animals were to be comparable.

The response to progesterone is shown in Table 1, where a highly significant increase in the quotient of secretion was given by 0·1 mgm. of progesterone.

Table 1. Secretion by the Glands of the Oviducts of Frogs and Toads in Response to Progesterone or an Extract of Ovulating Ovaries. Figures in Brackets Denote the Number of Animals in Each Group

Treatment	*Rana temporaria*		*Bufo bufo*	
	Quotient of secretion ± *S.E.*	*P**	Quotient of secretion ± *S.E.*	*P**
0·2 ml. arachis oil in lumen of oviduct	6·07±0·79 (12)	—	12·8 ±0·996	—
0·5 mgm. progesterone in lumen of oviduct	13·1±3·37 (10)	<0·03	—	
0·1 mgm. progesterone in lumen of oviduct	12·9±2·36 (7)	<0·01	40·1 ±5·32	<0·001
0·1 mgm. progesterone in dorsal lymph sac	—		41·02±5·18 (9)	<0·001
0·2 ml. ovary extract in lumen of oviduct	—		29·4 ±8·11 (3)	<0·05

* Values of *P* for comparison of experimental groups with arachis oil controls (using right-hand tail '*t*' test).

In the frog increasing the dose to 0·5 mgm. did not appear to promote further secretion. In the toad a very similar response was obtained whether the progesterone was injected directly into the oviduct or into the dorsal lymph sac.

In another series of experiments twelve toads were each injected with 1,000 I.U. of chorionic gonadotrophin ('Pregnyl') and killed 48 hr. later when the animals were in various stages of ovulation. The ovaries were removed, dried at 37° C. for 24 hr., then ground up in a mortar with sand and extracted with ether in a Soxhlet apparatus. 0·2 ml. portions of the clear oil obtained after evaporating off the ether were injected into the ligatured oviducts of ovariectomized and hypophysectomized toads as in the progesterone series. Table 1 shows that this extract gave a higher mean quotient of secretion compared with arachis oil controls, though there was considerable variation in the responses of the assay animals.

These experimental results agree closely with the more detailed work by Houssay and his co-workers on *B. arenarum*, and suggest that the production by the ovary during ovulation of a hormone having a stimulating action on the glands of the oviduct may be a general characteristic of the Amphibia.

Patricia D. B. Lodge
C. L. Smith

Department of Zoology,
University of Liverpool.

[1] Galli-Mainini, C., *Rev. Soc. Argent. Biol.*, **26**, 166 (1950).
[2] Houssay, B. A., *Rev. Soc. Argent. Biol.*, **26**, 185 (1950).
[3] Allende, I. L. C. de, *C.R. Soc. Biol. Paris*, **130**, 163 (1939).

PLANT PHYSIOLOGY

Response of Growing Plants to a Uniform Daily Rotation

Plants under various fixed conditions of light, temperature, and humidity have been turned continuously about a vertical axis at the rate of one revolution a day. The results suggest that the plants used are sensitive to the direction of rotation; a rotation which is clockwise when viewed from above inhibits growth, anti-clockwise direction stimulates growth. As the finding may be useful background to a study of the twining of plants, the initial results are being reported.

Cyclamen. In subdued daylight, or in artificial top lighting, plants which were made to rotate clockwise showed, after two or three days, a loss of turgidity in some of the leaves, which yellowed and died as the experiment continued. Plants turned anti-clockwise were as healthy and turgid as stationary ones.

Scarlet Runner Beans. Beans which normally twine anti-clockwise were found not to twine around their supports when growing plants and their supports were rotated anti-clockwise at one revolution a day under fixed side-lighting with tungsten lamps. Plants which were turned clockwise began to twine but lost turgidity, and some leaves yellowed and died. When these plants were transferred at an early stage of reaction to anti-clockwise movement they showed partial or full recovery.

Oats. Husked oats (variety Milford), were pre-chilled in tap-water at 5° C. for 48 hr. The seed was then sown, groove side down, on moist filter paper in a covered dish, and held at 20° C. in darkness. After 24 hr., germinated seedlings were transplanted on to 4-in. diameter perforated plastic holders over tap-water. Each holder and dish (48 seedlings per holder) was transferred to a completely dark, controlled-climate cabinet provided with a suitable set of turntables to give clockwise and anti-clockwise rotations. Cabinet temperature was controlled to 1° C. and relative humidity to 1 per cent. In successive experiments the controlled temperatures ranged from 25° C. to 28° C. and the relative humidity 85–95 per cent. After 5 days, the lengths of coleoptiles and roots were measured. In every experiment the mean lengths under each treatment were expressed as a percentage deviation from the mean of the stationary plants. The average deviations throughout nine experiments were as follows:

Clockwise	Coleoptiles	− 6·1 per cent
	Roots	− 8·6 ,,
Anti-clockwise	Coleoptiles	+ 5·6 ,,
	Roots	+ 10·2 ,,

In individual experiments the means of the clockwise-rotated sets of plants were always smaller than those of the stationary, and the anti-clockwise in all cases larger (for example, for coleoptiles—clockwise 42·7 ± 0·8 mm., stationary 46·3 ± 0·7 mm., anti-clockwise 48·2 ± 0·9 mm.).

The subject of diurnal rhythms as affecting the above is being considered.

R. L. Jones

Dominion Physical Laboratory,
Department of Scientific and
Industrial Research,
Lower Hutt,
New Zealand.

Energy-trapping Centres in Photosynthesis

In opaque specimens of packed *Chlorella* cells, the quantum yield of red fluorescence varies with the wave-length of the exciting light[1]. From a maximum value measured at the absorption peak at wave-length 675 mμ, decreases to about 70 per cent of this value occur at shorter wave-lengths, as shown in Fig. 1. In contrast, dilute suspensions with measured true absorption give a relatively constant yield, suggesting that competitive selective scattering and re-absorption losses are largely responsible for the yield decreases observed in opaque specimens.

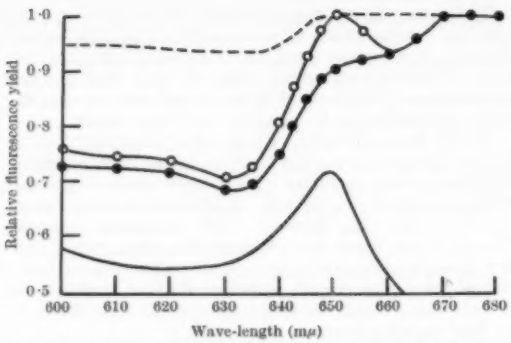
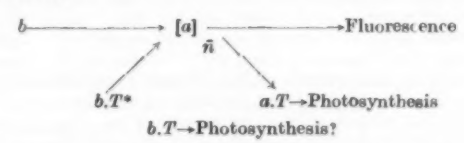


Fig. 1. Dependence of the relative fluorescence on the excitation wave-length and intensity in *Chlorella*. – – – –, Thin suspension; ○——○, opaque specimen (high intensity); ●——●, thick specimen (low intensity); ————, difference spectrum produced by intensity (ordinate × 2)

It has now been found that a part of the effect depends on the light intensity employed in yield determinations. High-intensity light produces a relatively more constant yield compared with low intensities, and the spectral dependence of the relative yields of *Chlorella* at different levels of illumination are included in Fig. 1. The absolute fluorescence yield at high-intensity excitation was 3–4 times greater than at low light levels. Since no changes in absorption or fluorescence spectra larger than 1 per cent occurred during these measurements, the difference spectrum corresponding to the selective increase in yield must be produced by a change in the effective fluorescence yield of a component pigment. The similarity in position of the difference spectrum maximum, shown in Fig. 1, to that of chlorophyll *b* *in vivo* suggests the involvement of this pigment. Before discussing the significance of this observation, it is necessary to consider the different situations of chlorophylls *a* and *b* in relation to energy transfer. Assuming that this process is responsible for energy migration to photochemically active trapping centres present in relatively small concentration, then these centres must contain a non-fluorescent pigment with an absorption band overlapping the fluorescence band of chlorophyll *a*. Changes in the trapping efficiency, accompanied by inverse changes in fluorescence yield, can occur in two ways if the geometry of the system remains constant. A change in absorption in the trapping pigment could reduce energy acceptance, or alternatively, the trapping pigment can become fluorescent. The absence of considerable absorption and fluorescence spectral changes means either that the trapping pigment is present in such small concentration that its absorption changes are undetected or that it is chlorophyll *a* itself or a spectroscopically similar molecule. The reported similarity of the fluorescence and delayed-light spectra[2] observed in *Chlorella* supports the involvement of chlorophyll *a*. On this latter basis, the simplest model for a trapping centre would be a complex between chlorophyll *a* and a colourless acceptor molecule, capable in its ground-state of quenching the chlorophyll singlet excited state, being itself converted to a metastable, chemically active form. Providing the ground-state interaction of the components is small, the associated spectral changes will be also small, as they are in chlorophyll *a* quenching by complex formation with aromatic nitrocompounds and quinones[3].

Detectable spectral shifts occur with chlorophyll *b* however, and some *in vivo* measurements of absorption changes in illuminated *Chlorella*[4] may be relevant in this connexion. During the life-time of the metastable form, the associated chlorophyll molecule could fluoresce normally. Calculation indicates that about 3 per cent of the total chlorophyll, complexed in trapping centres, would receive by resonance transfer at least 75 per cent of all absorbed quanta, the balance appearing as heat or fluorescence. In the trapping centres described, chlorophyll *b* might replace chlorophyll *a* in some instances. Few transfers among chlorophyll *b* molecules can occur *in vivo*; because of the relatively lower concentration of this pigment, and the large overlap of its fluorescence band with the absorption peak of chlorophyll *a*, excited chlorophyll *b* always transfers directly to chlorophyll *a*. As a consequence, that fraction of chlorophyll *b* which does not sensitize fluorescence must consist entirely of quenched molecules. This fraction corresponds to about 10 per cent of the whole absorption at 650 mμ, or to about 25 per cent of all chlorophyll *b*, which contributes roughly 40 per cent to the total absorption at this wave-length. The transfer processes occurring between the chlorophyll types can be shown in the following scheme:



where T and T^* represent the acceptor molecule in the ground and metastable states, respectively, and $\bar{n}$ is the average number of transfers among chlorophyll *a* molecules before trapping. A feature of this scheme is that at high intensities of excitation both types of chlorophyll *b* molecules sensitize chlorophyll *a* fluorescence and photosynthesis. The negligible overlap of chlorophyll *a* fluorescence with chlorophyll *b* absorption prevents the quenched fraction of this pigment from trapping chlorophyll *a* fluorescence. If this overlap is not negligible, however, a scheme requiring only chlorophyll *b* trapping centres could be proposed. The possibility that the chlorophyll *a* and *b* trapping centres may be shared or photochemically different is suggested by recent reports of the effect of supplementary light on photosynthesis[5]. In addition, the fall in photosynthetic yield in *Chlorella* which is observed at certain wave-lengths[6] and is usually attributed to inactive carotenoid absorption, could equally well be attributed to an inactive fraction of chlorophyll *b*, as was indeed suggested by Emerson.

This work was sponsored by the Office, Chief of Research and Development, U.S. Department of Army.

F. W. J. Teale

Department of Biochemistry,
The University,
Sheffield.

[1] Duysens, L. N. M., thesis, Univeralty of Utrecht (1952).
[2] Arnold, W., and Davidson, J. B., *J. Gen. Physiol.*, **37**, 677 (1954).
[3] Livingston, R., and Ke, C.-L., *J. Amer. Chem. Soc.*, **72**, 909 (1950).
[4] Strehler, B. [L., and Lynch, V., *Arch. Biochem. Biophys.*, **70**, 527 (1957).
[5] Emerson, R., and Chalmers, R. F., *Physiol. Soc. Amer.*, **11**, 51 (1958).
[6] Emerson, R., and Lewis, C. M., *Amer. J. Bot.*, **30**, 165 (1943).

BIOLOGY

An Automatic Fish Egg Counter

THE fecundity of fish has hitherto been generally determined by making direct counts of weight or volume sub-samples of the total gonad contents. These counts are then raised by the ratio of the total weight or volume of the gonad contents to the sample weight or volume.

This process is very time-consuming, and, in consequence, routine studies of the fecundity of large numbers of fish have not been practicable. While typical fecundity estimates have been obtained for a large number of fish species, using material classified by size and age, and collected in one or two seasons, no detailed long-term investigations have been conducted. Such investigations could be of considerable value in studies of the biology and population dynamics of fish populations where it is desired to know the changes in fecundity from year to year, the factors determining these changes, and the dependence of recruitment and strength of year-class on these changes.

To meet this need for routine measurement of fecundity (in the first instance of herring) an apparatus has been devised here. This makes rapid counts of all the eggs in a gonad, thus eliminating the tedious work of hand-counting, and also eliminating the errors of sub-sampling.

The apparatus is shown diagrammatically in Fig. 1. This shows that the eggs are drawn in a stream of water through a constricted tube, interrupting a beam of light which is directed through it. The light beam falls on the cathode of a photomultiplier tube, and the interruptions of the beam are recorded on an electronic counter.

The constricted tube, or counting chamber, has a minimum internal diameter of 1·53 mm., and it has been found that this is just large enough to pass the largest herring eggs without causing frequent stoppages.

The photomultiplier tube used is an 'R.C.A. type 931*A*' with nine dynodes, and the light source is a 2·2-V. 0·25-amp. lens-end flash-light bulb. The counter is a 'Panax type *D*554' which conveniently supplies the E.H.T. for the photomultiplier (roughly 200 μamp. at 1,000 volts) and an a.c. supply for the light source.

As will be seen from Fig. 1, a flow of bubble-free water is maintained from a constant-head tank through the counting chamber. Eggs to be counted are first cleared of all connective tissue and placed in water in the large glass funnel, and the stirrer speed is adjusted to bring them into suspension. The tap at the base of the funnel is then opened, and the eggs sink slowly downward and are swept through the counting chamber. The rate of sinking, and so the counting-rate, can be adjusted by varying the speed of the stirrer. In practice, a counting-rate of about 100 per sec. is found to be best. Attempts to increase this rate generally result in blockage of the system.

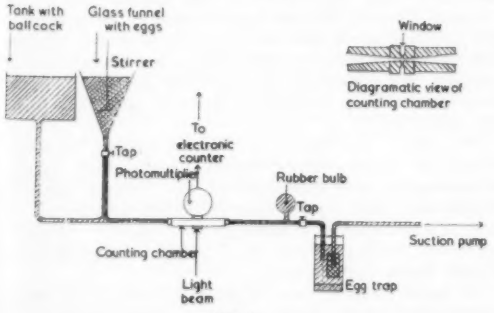


Fig. 1

A considerable number of trials have been made to gauge the accuracy and consistency of the counts. Typical sets of results are shown in Table 1, which gives small successive counts of samples of known size, and in Table 2, which shows successive counts of large samples.

Table 1

Sample	Actual number of eggs	No. recorded by counter in successive trials			
		1	2	3	4
1	521	521	519	525	523
2	778	775	777	771	770
3	507	505	505	505	503
4	808	805	803	801	800

Table 2

Sample	No. of eggs recorded by counter		
	1	2	3
1	19,088	19,128	19,101
2	25,093	25,135	25,274
3	39,494	39,513	39,596
4	43,106	43,150	43,279

These results show that the maximum discrepancy between counts and the maximum error on known samples does not exceed 1 per cent. It is thus felt that reliance can be placed on the method, and a programme of routine investigations of herring fecundity is being developed accordingly.

B. B. PARRISH
I. G. BAXTER
M. J. D. MOWAT

Marine Laboratory,
Aberdeen.

A Pale Hag-fish

DE GRAAF[1] and more recently Ewer[2] have described specimens of *Xenopus laevis* lacking hæmoglobin. In the course of an investigation on the physiology of the heart of the cyclostome *Myxine glutinosa*, I found on August 20 an animal about 25 cm. long which was remarkably pale, except in the caudal and dorsal regions, which were grey. Its blood appeared to have no pigmentation due to hæmoglobin except near the gills, where there were some small localized red areas. All the blood vessels were cream in colour as well as the heart, although the ventricle might have been pink (? myoglobin, cytochromes). The liver was dark green and after fixation in 8 per cent formol the principal dorsal vessels showed a light greenish coloration due perhaps to the destruction of hæmin compounds. As for the gall bladder, it did not appear greener than normal.

When first observed, the animal was in the dark in circulating sea water at 15° C. among a mixture of hag-fish, and showing as much vitality apparently as the other animals; it is particularly interesting to emphasize that this specimen had been caught at the same time as the others, two days before, in the Gullmars Fjord, Sweden, using G. Gustafson's technique, that is, by means of a large bottle contain-

ing some pieces of mackerel. Thus this animal, which is very poor in respiratory blood pigments, shows normal habits of feeding, proving that its olfactory organs were in good physiological condition. Hence the pathological state of the blood of this hag-fish leads one to ask what is the limiting role which could be taken by hæmoglobin in the life of *Myxine*.

My thanks are due to Dr. Swedmark, director of the Kristinebergs Zoologiska Station, Fiskebäckskill, Sweden, for providing many living hag-fishes.

B. Rybak

Laboratory of Zoophysiology,
University of Caen.

[1] De Graaf, A. R., *J. Exp. Biol.*, **34**, 173 (1957).
[2] Ewer, D. W., *Nature*, **183**, 271 (1959).
[3] Gustafson, G. J., *Ark. Zool.*, **28**, A, No. 2 (1934).

Interlaminate Figures of Sessile Barnacle Shells

Alessandri[1], Gruvel[2] and Davadie-Suaudeau[3], in their works on the cirripedes, have directed attention to figures or patterns in the shell plates of various species of sessile barnacles, and further work may prove these to be an important taxonomic characteristic for species identification; but this seems to have been somewhat neglected.

The usual method of identifying barnacles is often difficult due to the variability of the shell configurations and must frequently be followed by examination of the soft parts—a tedious undertaking. Even then, mistakes in naming have occurred, but work by Cornwall[4–6] on many specimens has shown that the 'interlaminate' figures are specific and constant for recent and fossil barnacles.

The following method of revealing the figures is a modification of the procedure using plaster of Paris as described by Cornwall. Whole specimens or plate fragments are embedded in 'N.H.P.' mounting plastic (obtained from North Hill Plastics, Ltd., London). Rings of tubular rubber, polythene or cardboard are cut to fit the size of the specimen to be mounted. The rings are placed on a glass plate to which is applied a thin coating of 'Vaseline' and the specimen carefully placed, basis downwards, within the ring. A small amount of the plastic powder ('N.H.P. 2031/19') is spread over and around the specimen and thoroughly wetted with the plastic fluid ('N.H.P. 1844'). When the first application of liquid has been absorbed a fresh portion of powder, followed by more liquid, is added. This procedure is repeated until all, or as much as is thought necessary, of the specimen is covered. Leave to dry and harden (30–60 min.). When complete specimens are to be mounted it is advisable to fill the shell cavity with plastic by turning the mounting ring over and repeating the above process. When ready, the embedded specimen is extruded from the ring or, if this proves impossible, it is cut away and is ready for grinding and polishing.

It is necessary, in order to show the figures to their best advantage, to ensure that the surface to be examined is as flat and even as possible. A coarse grinding is done on a glass plate with wet 320 silicon carbide powder followed by a similar grinding on a second plate with 700 powder. Grinding should be done until the parietal canals are visible. This can be controlled by examining the ground surface at intervals with a hand lens. After grinding, the surface is given a rinse with water to remove any adhering carbide particles and then dried by pressing on to a filter paper. Polishing is done with diamond paste on a chamois leather followed by a final buffing on a dry chamois leather.

The figures are best seen at a magnification of between 20 and 50 with the light from the top and slightly to the side of the mounted specimen. A few drops of water or benzyl alcohol[5,6] run on to the prepared surface between a cover-slip will often help reveal figures which tend to 'disappear' when the mount is dry. Orienting the specimen to the light is also a help in showing up details of the figures. One of the great advantages of the method outlined above is that the mounted specimen can be dissolved out of the plastic mount by steeping in chloroform, when it can be retrieved intact except for the small portion which has been ground away.

Certain species such as *Balanus balanoides* and *Elminius modestus* have not shown any figures in preparations from the basis. This may be due to the fact that these species do not have any parietal canals (Crisp, D. J., personal communication). It may be that such species will show figures if preparations are made from the ala of the wall plates[6].

An atlas of the interlaminate figures from identified barnacle specimens would be of great value for the further identification of species and would help in furthering this work.

R. Read

43 Holly Terrace, Hensingham,
Whitehaven, Cumberland.
Dec. 12.

[1] Alessandri, Guido de, *Boll. Soc. Geol. Italiana*, **13**, 3, 234 (1895).
[2] Gruvel, A., "Monographie des Cirrhipedes ou Thecostraces" (1905).
[3] Davadie-Suaudeau, (Madam) Claude, Bull. du Service de la Carte Géologique de l'Algérie, Ser. 1, Paléontologie, No. 14 (1952).
[4] Cornwall, I. E., *J. Paleontol.*, **30**, 3 (1956).
[5] Cornwall, I. E., *Canad. J. Zool.*, **36**, 79 (1958).
[6] Cornwall, I. E., *Canad. J. Zool.*, **37**, 401 (1959).

Seed Incompatibility in *Primula*

When diploid and autotetraploid plants of *Primula veris* are artificially crossed reciprocally, fertilization occurs and seed is set, but the seed is bad and germination is nil; the same is true for *Primula vulgaris* and *P. elatior*. Similar seed incompatibility (as it may be called) between diploids and their colchicine-induced autotetraploids has been reported in several other genera of flowering plants, for example, *Gossypium*[1] and *Galeopsis*[2]; it is associated with post-fertilization breakdown of the seed tissues, in particular the endosperm. On the other hand, in some genera, for example, *Beta*[3], the crosses are easily made and viable triploids can be produced in large numbers. The reasons for these different types of behaviour are not known, but they are probably connected with differences in the nature and mode of functioning of the tissues concerned in the nutrition and protection of the embryo; it is worth noting that these tissues (the endosperm and the maternal nucellus and endothelium) generally differ in genetic constitution both from the embryo and also from one another.

The seeds produced in the reciprocal diploid–tetraploid crosses in *Primula* are of two types, according to the direction of the cross; similar contrasting seed types are found in a number of diploid interspecific crosses in this group[4]. This is shown in Table 1, where the *P. elatior–P. veris* cross is used as an illustration.

In these crosses, type *A* seed is very small, with abnormally thick integuments, a small amount of

Table 1

♀ parent		♂ parent	Seed type
P. veris (2*n*)	×	*P. veris* (4*n*)	*B*
P. elatior (2*n*)	×	*P. veris* (2*n*)	*B*
P. veris (4*n*)	×	*P. veris* (2*n*)	*A*
P. veris (2*n*)	×	*P. elatior* (2*n*)	*A*

endosperm, which usually degenerates, and a small, often persistent embryo. Type *B* seed is of normal size; the integuments are thin, and the endosperm and the minute embryo degenerate soon after fertilization. Both types of seed are usually nearly empty at maturity and often the seed walls collapse; (very rarely a viable diploid hybrid may be produced from the *veris–elatior* (type *A*) cross[5]). The classification of the seeds into types *A* and *B*, originally based on seed dissection, is fully supported by detailed embryological investigation of their development and breakdown; so that the two pairs of crosses described in Table 1 may be regarded as analogous. In one, seed incompatibility is presumably conditioned by the qualitative gene differences between the two diploid species, and in the other by the quantitative gene differences between the diploid and the autotetraploid.

In previous work on the diploid crosses[4], this analogy led us, following the work of Stephens on *Gossypium*[1] and Howard on *Nasturtium*[6], to think of the genomes of the species in terms of 'genetic strength', and to assign to each genome a numerical value, the genetic value. The results of reciprocal interspecific crosses could then be interpreted in terms of the ratio of the genetic values of the various tissues of the seed, these ratios being different in any one pair of reciprocal crosses. The ratio of the genetic value of the endosperm to that of the maternal tissues has in fact been thus used in interpreting segregation, in interspecific hybrids, of the genes controlling seed incompatibility[7]. In this interpretation, the genetic value of *P. elatior* was set at unity and that of *P. veris* at 1·8, to conform with the fact that the degree of incompatibility in the *elatior–veris* crosses was slightly less than that in diploid–autotetraploid crosses; in the latter, it is assumed that the ratio of the genetic values of the parents is always 1 : 2.

The analogy between the two types of cross can be tested by making reciprocal crosses between the autotetraploid *P. elatior* with a genetic value of 2, and diploid *P. veris* with a genetic value of 1·8. The values of the parents are thus approximately equal, so that theoretically the cross should produce good seed and viable triploid hybrids in either direction; the seeds should tend to type *A* when *P. elatior* (4*n*) is the female parent and to type *B* in the reciprocal. In fact the cross *P. veris* (2*n*) ♀ × *P. elatior* (4*n*) ♂ gives a fair yield of good seeds; germination percentages up to 67 per cent have been recorded, and the hybrids are triploid. Embryological study shows that these seeds are, as expected, of a moderate type *B*; there is a fair amount of endosperm, which shows some abnormalities. On the other hand, the reciprocal cross, *P. elatior* (4*n*) ♀ × *P. veris* (2*n*) ♂, produces rather small seeds, practically empty at maturity, which embryological investigation shows to be of an unexpectedly extreme type *A*, with very thick integuments; and no viable hybrids are produced.

It may be concluded that some at least of the genetical differences which control seed incompatibility between *P. elatior* and *P. veris* are analogous to those between diploids and autotetraploids, and are of a quantitative nature; thus they may be duplications, either of genes or of small chromosome segments. Over and above these differences, however, are others of a qualitative kind; the fact that the reciprocal crosses between *P. elatior* (4*n*) and *P. veris* (2*n*) give such strikingly different results, even though their genetic values are approximately equal, suggests that the qualitative genetic differences may have a cytoplasmic as well as a nuclear component.

A full account of this work is in preparation.

D. H. Valentine
S. R. J. Woodell

Botany Department,
Durham Colleges,
University of Durham,
and
Botany Department,
University of Oxford.

[1] Stephens, S. G., *J. Genet.*, **44**, 272 (1942).
[2] Håkansson, A., *Hereditas*, **38**, 425 (1952).
[3] Rasmusson, J., *Nord. Jordbrugsforsk.* (1948).
[4] Valentine, D. H., *Symp. Soc. Exp. Biol.*, **7**, 146 (1953).
[5] Valentine, D. H., *New Phytol.*, **50**, 383 (1952).
[6] Howard, H. W., *J. Genet.*, **48**, 111 (1947).
[7] Valentine, D. H., *New Phytol.*, **55**, 289 (1956).

Induction of Traps of Nematophagous Fungi using *Panagrellus redivivus*

Following the revived interest in biological control of plant-parasitic nematodes through use of nematode-trapping fungi, much attention is being devoted to collection, identification and economic utilization of these ubiquitous microfloræ.

One conventional method of isolating these forms involves addition of suitable mycogenous material to maizemeal agar. Following a suitable period of growth, during which time fruiting structures are produced, nematode-trapping devices such as adhesive mycelial meshes or knobs, and constricting rings may be in evidence. In such instances the fungus either has been able to form such traps exclusive of the presence of nematodes or else a suitable number of free-living nematodes were present in the mycogenous material and thus stimulated trap formation. In certain cases, however, an external source of nematodes must be utilized in order to provide sufficient impetus for these fungi to exhibit their predacious devices.

Drechsler[1] used additional putrescent material such as friable leaf mould for supplying nematodes to cultures. Comandon and de Fonbrune[2] used sterilized free-living soil nematodes, while other workers employed animal parasitic nematodes[3,4]. Plant-parasitic eelworms such as *Heterodera rostochiensis*[5] and *Ditylenchus dipsaci*[6] also have been used successfully. Duddington[7] found that enormous numbers of free-living nematodes could be reared on rabbit dung infusion agar, but admitted that "One of the major difficulties in working with predacious fungi is maintenance of adequate stocks of eelworms for use when they are required"[8].

During the past two years the nematode *Panagrellus redivivus* (L.) Goodey, 1945, has been utilized with great success for producing fungal traps in cultured nematophagous hyphomycetes. Following growth of moulds from suitable mycogenous material implanted on maizemeal agar, either a water suspension or a writhing mass of *Panagrellus* is introduced

into agar plates. Within a day or two fungal trapping structures usually are manifested in legion. Since reproduction of this nematode is continual and prolific, worms of many sizes are produced, allowing even the smallest of traps to become functional. An adequate supply of eelworms can be maintained constantly on cellulose sponges drenched with potato broth[9] or by use of the oatmeal culture method of Cheo and Tarjan[10]. The latter method, which I have used successfully for several years, involves culture of the worms in an oatmeal–cation exchange resin medium. Once the animals have multiplied sufficiently they will form thickly populated, culture medium-free, curd-like masses on sides of the culture vessel above the medium, which can be readily scraped off for introduction into agar plates. Oatmeal cultures in which worms are established can be stored at 2–5° C. When needed, cultures are warmed to room temperature, resulting in accumulation of nematodes on sides of the culture vessel as referred to above. *Panagrellus* populations can be maintained for years by refrigeration, after establishment on test-tube agar slants.

Armen C. Tarjan

Florida Citrus Experiment Station,
Lake Alfred, Florida.
Oct. 30.

[1] Drechsler, C., *Biol. Rev.*, **16**, 269 (1941).
[2] Comandon, J., and de Fonbrune, P., *C.R. Soc. Biol., Paris*, **129**, 619 (1938).
[3] Deschiens, R., *Bull. Soc. Path. Exot.*, **32**, 394 (1939).
[4] Schmidt-Hoensdorf, F., and Ehrentreich, F., *Ztrlbl. Vet.*, **4**, 390 (1957).
[5] Hutchinson, S. A., and Mai, W. F., *U.S. Dept. Agric. Plants Dis. Rpt.*, **38**, 185 (1954).
[6] Duddington, C. L., "The Friendly Fungi", 173 (Faber and Faber, London, 1956).
[7] Duddington, C. L., *Trans. Brit. Mycol. Soc.*, **38**, 100 (1955).
[8] Duddington, C. L., *Bot. Rev.*, **21**, 432 (1955).
[9] Taylor, A. L., Feldmesser, J., and Feder, W. A., *U.S. Dept. Agric. Plant Dis. Rpt.*, **41**, 527 (1958).
[10] Cheo, P. C., and Tarjan, A. C., *Phytopath.*, **45**, 184 (1955).

A Mating-type System in an Acellular Slime-mould

An investigation of the life-cycle of the acellular slime-mould (myxomycete) *Physarum polycephalum* has revealed that a mating-type system exists in this organism, and analysis of this system has been carried out. The existence of mating-types in myxomycetes has previously been suggested by Skupienski[1] as a result of his work on *Didymium nigripes* and *D. difforme*, but the situation was not fully investigated.

The spores of *Physarum polycephalum* release uninucleate amœbæ which, after a variable number of divisions, fuse in some unknown way to form the acellular, multinucleate plasmodium. The plasmodium increases in size and, under suitable conditions, produces spores.

A plasmodium, kindly supplied by Dr. Rusch of the University of Wisconsin, produced spores from which a series of cultures was started. Ten out of twenty-five such cultures grown from groups of spores produced plasmodia, but none of twenty cultures grown from single spores did so. These results are similar to those of Skupienski with *D. difforme*.

Groups of amœbæ from ten single isolated spores were used to set up 55 different cultures: 10 of these cultures were started with amœbæ of the ten groups taken separately, and forty-five started with amœbæ from all possible combinations of the ten groups taken two at a time. The incidence of plasmodium formation among these cultures was plotted on a chart, and the results indicated that a simple two-type (+/−) mating system was in operation (Fig. 1). The test was repeated twice, and in the combined results of all three tests, plasmodium formation occurred in twenty-one of the twenty-five cultures in which it was expected on the basis of a +/− system, but not in any of the twenty combined-group cultures in which it was not expected or in any of the ten single-group cultures. The failure to form plasmodia in four of the cultures in which it was expected could be due to factors other than mating-type, since it was noticed in all experiments that plasmodium formation is dependent also upon a number of external conditions, particularly humidity.

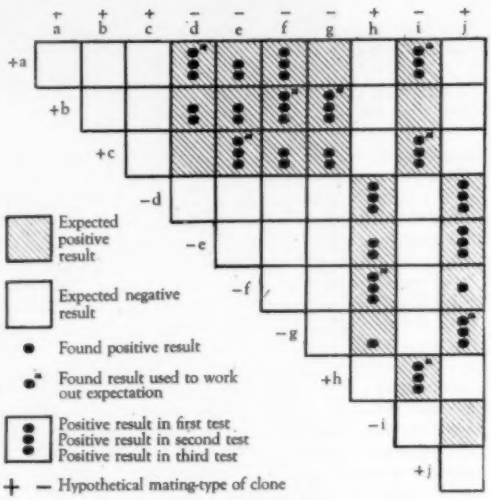


Fig. 1. Tests of the mating-type system. The ten clones were denoted by the letters *a*–*j*. Each square in the chart represents a culture. Forty-five cultures were started with amœbæ of the ten clones taken in all possible combinations of two at a time, and ten control cultures were started with amœbæ of the ten clones taken separately. The mating-type + or − was assigned to each clone on the basis of nine of the results of the first test. These nine results are marked as shown in the key

It may be concluded from these results that plasmodium formation takes place only when amœbæ of different mating-type meet, that only two mating-types occur in the cultures investigated, and that a spore produces a clone of amœbæ of one mating-type only.

Investigation of the inheritance of the mating-types gave the following results. Fifty clones of amœbæ were established, each derived from a single spore of a plasmodium produced by one of the combinations of two clones described above. Each of these fifty first-generation clones was then tested for plasmodium formation with each of the two parent clones. Fifteen of the first-generation clones formed plasmodia with one parent clone only, ten with the other parent clone only, and twenty-five with neither parent. These results are consistent with the hypothesis of a simple +/− system. The first-generation clones tested two at a time among themselves gave further results also consistent with this hypothesis. However, many of the combinations have failed to produce

plasmodia in repeated tests. This suggests, as could be expected, that inherent factors other than mating-type may affect mating and plasmodium formation. While the results are consistent with a two-type mating system, they are not consistent with any of the multifactorial mating-type systems well known in other micro-organisms.

JENNIFER DEE

Department of Genetics,
The University,
Glasgow.

[1] Skupienski, M. F. X., *C.R. Acad. Sci., Paris*, **167**, 31 (1918); **182**, 150 (1926).

MICROBIOLOGY

Mode of Action of Hair-loosening Organisms

CONSIDERABLE research has been devoted to the development of enzymes for replacing the normal putrefactive techniques for the loosening of wool and hair fibres on animal pelts. Detailed knowledge of the tissues responsible for anchoring the fibre in the skin is therefore essential.

Examination of fibres obtained from skins which have been exposed to bacterial attack shows that the follicle bulb has been completely digested, leaving a tapered root of exposed cortical cells. It is considered that digestion of the root bulb permits the fibre to be readily withdrawn from the skin and that the organisms responsible penetrate down the follicle to attack the soft keratin.

In recent work, however, we have related the appearance of the follicle to the force necessary to withdraw fibres from pelts and have observed that, when the depilation load has fallen to the level at which the fleece is normally pulled from the skin, the whole follicle is removed intact with the fibre. At a later stage of putrefaction, although the depilation load is virtually unchanged, the follicle is rapidly digested to give the typical tapered appearance.

Fig. 1 shows a bundle of intact follicles on fibres pulled as soon as the depilation load had fallen to a level suitable for pulling.

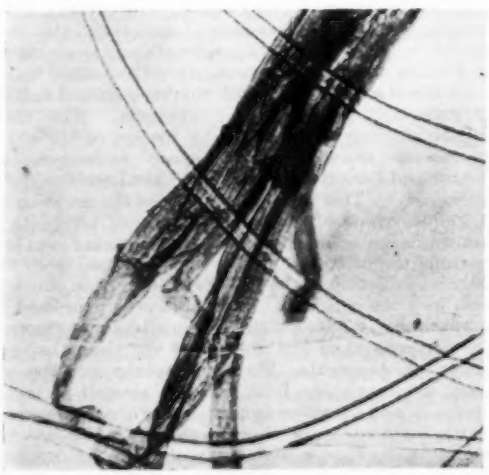


Fig. 1

It seems, therefore, that the bacteria penetrate from the flesh side of the skin to proliferate around the follicles, and that the most significant feature of fibre loosening is the digestion of material in these regions. Whereas efforts have, in the past, been concentrated on the degradation of the soft keratin, the production of an enzyme designed to digest the dermal material immediately adjacent to the follicle would seem likely to result in a greater yield of undamaged fibre.

K. J. WHITELEY

School of Wool Technology,
University of New South Wales,
Kensington, N.S.W.

Taxonomy of Yeasts

ON different occasions, when studying the classification of yeasts, I have been able to appreciate how little consistency there is in the taxonomic characters in use. Recently, I have met with fresh anomalies which, in my opinion, make a complete revision of the present system of classification of yeasts unavoidable.

(*a*) From the aqueous solution separated from oil during the manufacture of olive oil, in Spain named 'alpechín', a yeast culture was isolated, *Ac* 11, which is a characteristic *Zygosaccharomyces*, able to produce vigorous alcoholic fermentation and which ferments glucose, galactose, saccharose, melibiose and raffinose completely, and does not ferment or assimilate maltose or lactose. In accordance with the classification of Lodder and Kreger-van Rij[1], this yeast could be considered as a *Sacch. microellipsodes*; however, as to the organism described by Lodder and Kreger-van Rij as a *Sacch. microellipsodes*, is it truly a *Saccharomyces*? The Dutch authors admit that they have not been able to observe spores in the strain under study, and neither could Stelling-Dekker in 1931; moreover, Kudriavtzev[2] categorically denies that the organism is a *Saccharomyces*, not because of its inability to sporulate, but because of its morphology, its weak fermentation of the carbohydrates, etc.; and states that, in his opinion, its place in the classification system is not yet sufficiently clear. Therefore, it is possible that our organism *Ac* 11 corresponds to a *Saccharomyces* species which has not yet been described, for which I propose the name of *Sacch. malacitensis* (from Málaga, the province from which I received the sample of 'alpechín').

Moreover, I had earlier obtained a hybrid[3] crossing *Sacch. oxidans* (ferments glucose, saccharose and raffinose 1/3) and *Sacch. oleaceus* (ferments glucose, galactose, melibiose and raffinose 1/3), to which I gave the symbol *H* 7–4–1; this has a fermentation spectrum identical with that of *Sacch. malacitensis*. Though there are notable differences between the morphology of *H* 7–4–1 and *Sacch. malacitensis*, it would be reasonable to suppose that, were the origin of *H* 7–4–1 unknown, it would be classified as a *Sacch. malacitensis* strain. However, in biology it is inconsistent to consider as a new species an organism obtained by hybridation with other diverse organisms, since a species includes all the potentially interbreeding individuals, the descendants of which are also potentially interbreeding. Therefore the doubt arises whether the present separation of species in the genus *Saccharomyces* is admissible, based as it is fundamentally on the simple difference of fermentation of only one carbohydrate.

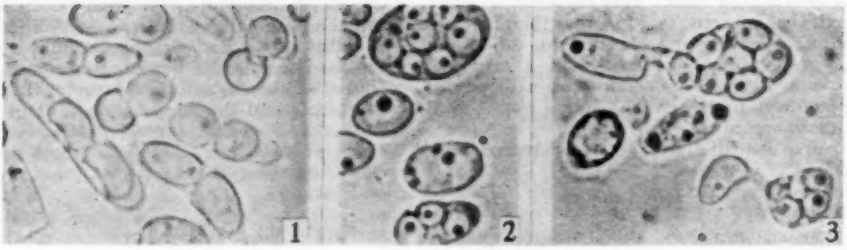


Fig. 1. *Pichia membranaefaciens*, strain *VB* 65 (*T* 109). Asci with bean-shaped spores. From *MA* 29 sporulation medium, after 5 days at 25° C. Unstained. (× 2,000)

Fig. 2. *Pichia membranaefaciens*, strain *Ac* 40. Asci with three and seven spores. From the pellicle formed in malt extract, after 3 days at 25° C. Unstained. (× 2,000)

Fig. 3. *Pichia membranaefaciens*, strain *Ac* 40. Asci with four and seven spores formed after heterogeneous conjugation. From the pellicle formed in malt extract, after 3 days at 25° C. Unstained. (× 2,000)

(*b*) From a sulphurized grape must which began to ferment spontaneously four months after preparation, among other yeasts a culture, *VB* 65 (*T* 109), was isolated, which was a characteristic strain of *Pichia membranaefaciens* but possessed the peculiarity that, as well as the normal spores, it also formed kidney-shaped spores; such spores have never been mentioned in the descriptions of the genus *Pichia*, not even in the very modern one by Phaff[4]. As a rule, great importance is given to the form of the spores as a taxonomic characteristic; for example, Kudriavtzev[2] created the genus *Fabospora* on the basis of its bean-shaped or reniform spores, and established a relation between this spore form and its weak ability for fermenting carbohydrates. Wickerham[7] also, on establishing his new genus *Dekkeromyces*, conceded special importance to the spore form. It appears very difficult to separate my organism from the species *Pichia membranaefaciens*, in which case the taxonomic value of the spore form will be open to controversy.

(*c*) From samples of 'alpechín' obtained from two different parts of the province of Badajoz, two yeast cultures were isolated, *Ac* 40 and *Ac* 47, which are two strains of *Pichia membranaefaciens*; although *Ac* 40 forms only a rather primitive pseudomycelium in anaerobiosis, the new definition of the genus *Pichia* given by Phaff[4] admits species without or with a reduced pseudomycelium. These two strains develop a smooth pellicle in malt after three days at 25° C. and form a large number of asci with 1–4 spores, as well as others with 5, 6 and 7 spores; asci are formed without immediately preceding conjugation or after heterogamous conjugation. These asci have not been mentioned in the genus *Pichia*, so that a revision of the diagnosis of *Pichia* is necessary.

(*d*) From different samples of 'alpechín' obtained from 22 places in different regions of Spain, 28 strains of *Saccharomyces oleaginosus*[5] were isolated. One of these strains, which has characteristics coinciding completely with those typical of the species, nevertheless differs from the remainder in its positive assimilation of potassium nitrate. According to Lodder and Kreger-van Rij[1] and Wickerham[6], yeasts which reproduce asexually exclusively by budding, form 1–4 ascospores per ascus and assimilate nitrate, should be included in the genus *Hansenula*. Nevertheless, in view of the observations mentioned, I doubt whether the assimilation of potassium nitrate can be regarded as a taxonomic characteristic of importance.

A detailed account of this work will be published elsewhere.

Juan Santa María

Sección de Bioquímica,
Instituto Nacional de
Investigaciones Agronómicas,
Madrid.

[1] Lodder, J., and Kreger-van Rij, N. J. W., "The Yeasts: a Taxonomic Study" (North-Holland Pub. Co., Amsterdam, 1952).
[2] Kudriavtzev, V. I., "Sistematica Drozhzhey" (Academy of Sciences, Moscow, 1954).
[3] Santa María, J., Ann. Inst. Nac. Invest. Agronómicas (in the press).
[4] Phaff, H. J., *Ant. Leeuwenhoek. J. Mic. Serol.*, **22**, 113 (1956).
[5] Santa María, J., *Bol. Inst. Nac. Invest. Agronómicas*, **38**, 301 (1958).
[6] Wickerham, L. J., U.S. Dept. Agric. Tech. Bull. No. 1029 (1951).
[7] Wickerham, L. J., *J. Bact.*, **71**, 290 (1956).

Permeability of Dormant Bacterial Spores

The frequent assumption that bacterial spores are impermeable and thereby metabolically dormant, unstainable and resistant to environmental stress has not heretofore been tested by experiment. We have attempted to examine this problem using clean, dormant, thermostable spores of a common aerobic bacterium, *Bacillus cereus* strain terminalis.

The organism was grown in a liquid semi-synthetic medium to essentially complete and synchronous sporulation[1]. After sporangial autolysis, the free spores were harvested by centrifugation or foam separation and washed repeatedly with distilled water to demonstrable removal of stainable cells and debris, catalase and germinating compounds. The total uptake of exogenous solutes by masses of the clean spores was assessed by the 'space' technique, the details and formulations of which have been described previously[2]. This procedure involves the addition of a known volume and concentration of solute to a known large mass of cells; after mixing and repacking the cells, the uptake is determined from the degree of dilution of the solute resulting from penetration into the cell pack. This uptake is expressed as a space value (S), which is defined as the fraction (or percentage) of the volume of the pack that the solute can penetrate. However, uptake into the cell pack involves space both between as well as inside the cells, and therefore correction for the interstitium must be made. With a non-penetrating macromolecule, such as a high molecular weight dextran, the intercellular space (S_{in}) for spores centrifuged at 17,000g for 30 min. was about 10 per cent but

needed to be determined in each experiment. After appropriate allowance for this value, the uptake is expressed as a corrected space value (R), which is defined as the fraction (or percentage) of the volume of the cells themselves that the solute can penetrate.

Test of the impermeability hypothesis was made initially with glucose employed in a concentration of 10 mgm./ml. in distilled water at pH 6·8 and 0° C. with a 15-min. exposure period. After allowance for the intercellular space, the corrected mean glucose space (R) was found to be about 40 per cent whether measured isotopically, gravimetrically or by carbon combustion (Table 1). This uptake of glucose was essentially independent of temperature, pH, solute concentration, period of exposure and presence of other solutes or sodium azide and could be completely eluted with water. Thus, the observed uptake of glucose could not be accounted for by surface adsorption, exchange with internal glucose or metabolic removal. Rather, there appeared to be a net diffusional entry into a region occupying 40 per cent of the spore volume.

Table 1. Uptake of Glucose by Dormant Spores

Analytical method	No. of experiments	R (%)		
		Range	Mean	S.D.
Gravimetric	8	31–47	40	1·4
Isotopic	4	34–51	40	5·7
Carbon combustion	7	34–47	42	3·9

If so, then other small molecules could be expected to permeate dormant spores to much the same extent. Indeed, a series of representative compounds that included organic acids, amino-acids, sugars and sugar alcohols gave space values approximating that for glucose (Table 2). Two types of compounds provided notable exceptions. Compounds such as adenosine gave variably high space values, and elution was possible only at the isoelectric point, suggesting some adsorptive uptake. Compounds such as ethylene glycol and urea gave constantly high space values (up to 57 per cent) which appeared to be real and possibly associated with lipid solubility. Murrell and Scott[3] have demonstrated that deuterium solution can mix freely with the total water volume of bacterial spores. We are now attempting to confirm, by means of the space technique, this seemingly complete permeation of water into the spore.

Table 2. Uptake of Various Solutes by Dormant Spores

Solute	R	Solute	R
Pyruvate	0·42	Glucose	0·40
Succinate	0·44	Ribose	0·41
Citrate	0·41	Maltose	0·44
DL-Alanine	0·32	Raffinose	0·42
DL-Valine	0·40	Erythritol	0·42
L-Lysine	0·42	Glycerol	0·44
L-Aspartate	0·41	Ethylene glycol	0·53
Adenine	1·10	Propylene glycol	0·53
Adenosine	0·76–1·2	Urea	0·57

Uptake of larger molecules was determined with a graded molecular weight series (62–2,000,000) of polyethylene glycols and dextrans. Space values for these two compounds were comparable and diminished linearly through a hundred-fold increase in molecular weight, suggesting that the surface structures of spores into which exogenous solute penetrates have the equivalent of pores which either vary in size or become progressively smaller with depth. A minimum uncorrected space value of about 10 per cent occurred with dextrans of molecular weight 100,000 or greater; this constant value was concluded to represent the intercellular space.

Concurrent and previous studies[1] on structural differentiation and associated physiological changes have intimated that diffusional uptake is limited to the multilayered thick coat of the spore. Two internal membranes, the cortex and the contiguous inner membrane, appear to be formed at the same time that the core, apparently, of a maturing spore becomes light-refractile and resistant to stains. A mature spore with a fully formed cortex actually takes up stains, as measured with the space technique, and can be seen to absorb stain in its peripheral structure. Furthermore, such a spore can be totally stained after it has been crushed, indicating that the core is not chromophobic but that a barrier shielding it has been broken. Attempts also were made to localize the observed uptake by comparing average R values with direct measurements of spore-coat space. Calculations from measurements on electron micrographs of medial ultra-thin sections indicated that the volume occupied by spore membranes external to the core is approximately 40 per cent of the total spore volume, assuming spores to be ellipsoids of revolution and the spore structure to be of uniform density. This apparent localization of solute permeation in the peripheral membranes of dormant spores is being investigated further.

Detailed reports of these findings will be published elsewhere.

We acknowledge appreciatively the technical assistance of L. Jo-Ann Cherry and Sara S. DeLong. The work was supported by grants from the U.S. Public Health Service (*E*619 *C*4-*C*6) and the Michigan-Memorial Phoenix Project of the University.

Samuel H. Black
R. E. MacDonald
Tadayo Hashimoto
Philipp Gerhardt

Department of Bacteriology,
University of Michigan,
Medical School,
Ann Arbor, Michigan.

[1] Hashimoto, T., Black, S. H., and Gerhardt, P., *Canad. J. Microbiol.* (in the press).
[2] MacDonald, R. E., and Gerhardt, P., *Canad. J. Microbiol.*, **4**, 109 (1958). See also Britt, E. M., and Gerhardt, P., *J. Bacteriol.*, **76**, 288 (1958).
[3] Murrell, W. G., and Scott, W. J., Seventh Int. Congr. Microbiol., Absts., 26 (1958).

Decontaminating Tissue Cultures infected with Pleuropneumonia-like Organisms

The contamination of tissue culture cell lines by pleuropneumonia-like organisms has become an increasingly important problem[1–4]. The contamination itself is insidious and cannot be detected ordinarily by gross changes in the appearance of the media macroscopically or microscopically. The tissue culture cells themselves, when observed microscopically, may or may not exhibit slight cytopathology as evidenced by intracytoplasmic granularity. Properly stained preparations may reveal intracytoplasmic particles, presumably the contaminating pleuropneumonia-like organisms[5–7].

At present it is not understood how these organisms gain entrance to cultures, although speculation that

they represent *L* forms of bacterial contaminants seems to provide the most likely explanation[4]. Most pleuropneumonia-like organisms are resistant to penicillin but susceptible in varying degrees to streptomycin and certain of the broad-spectrum antibiotics[8,9]. Certain evidence indicates that the presence of high concentrations of antibiotics to which pleuropneumonia-like organisms are sensitive drives these organisms into intracellular residence, and that when the antibiotic concentration drops below a critical level (due to long incubation at 37° C. or extremes of *p*H) the organisms are free to reappear in the supernatant fluids. This cycle of appearance and disappearance of pleuropneumonia-like organisms in tissue culture fluids containing antibiotics must be considered when isolations of organisms are attempted from tissue cultures.

Since routine sampling of tissue culture media components including serum has not as yet revealed pleuropneumonia-like organisms, another explanation for the source of contamination must be considered. The suggestion that cell lines have been contaminated from the tissue of origin is probably not valid since: (1) routine sampling of primary tissue cultures has not revealed these organisms and (2) studies of HeLa cell cultures have not substantiated interpretations favourable to the hypothesis. The widespread prevalence of pleuropneumonia-like organism contamination of HeLa cell cultures and the frequent occurrence of pleuropneumonia-like organisms in the urogenital tract had been regarded as evidence that contamination occurred when the cell line was first isolated. However, routine testing of a number of HeLa sublines in 1953, when HeLa was first generally available, always gave negative results[5]. The techniques used in the attempted isolations at that time were the same as those used recently which resulted in positive isolations[4].

Accordingly, another explanation has been offered, that the pleuropneumonia-like organisms probably represent *L* forms of bacterial contaminants[4]. A complete list of the kinds of environmental trauma that can induce bacteria to revert to *L* forms is lacking. It is known, however, that agents like glycine, raffinose, salts of light metals, carboxymethoxylamine, chloromycetin, cold, X-rays, phage, glycerol and most of all penicillin, can cause this reversion[10]. This list includes a number of reagents that are used routinely in the tissue culture technique. Therefore the hypothesis is proposed that the *L* forms present in tissue cultures were induced by the action of penicillin, and also perhaps by glycine, salts of light metals, and glycerol, on bacteria. This process occurs when breaks in aseptic technique allow for the presence of contaminating bacteria which, under the influence of one of these reagents, revert to the corresponding *L* form.

A method for decontaminating pleuropneumonia-like organism (or *L* form) contaminated tissue cultures has been developed and is based on the differential heat lability of the pleuropneumonia-like organisms and the tissue culture cells. It has been found that if tissue culture cell lines, such as HeLa, *L* cells, *WISH* (a human amnion line), Fernandes amnion, and a human renal line, have been either naturally or artificially contaminated with pleuropneumonia-like organisms and are then held at 41° C. for about 18 hr., they remain negative for pleuropneumonia-like organisms indefinitely. The pleuropneumonia-like organisms are very heat-labile. Some strains are killed at 45° C. for 15 min., others succumb at 45° C. for 30 min.[11]. Tissue culture cells held at 45° C., in general, do not survive, yet when incubated at 41° C. for a long period of time differential survival is achieved—the pleuropneumonia-like organisms are killed and the tissue culture cells survive.

This hyperthermic treatment of cell lines is not without some effect on the cells, and tolerance to 41° C. varies from cell line to cell line, although they all apparently will survive[12]. HeLa at 41° C. has a tendency toward arborization, withdrawal of processes, some rounding and cytoplasmic vacuolization. However, this is by no means fatal to the culture, and once the cells are placed at 37° C. after treatment, recovery is almost always assured. Confirmation of the heat lability of two tissue culture pleuropneumonia-like organism contaminants was obtained when these strains were found to be killed when grown out on agar and then subjected to 41° C. for 18 hr. It is recommended that for optimum simplicity the heat treatment be done in air, since if a water-bath is used it will necessitate complete submersion of the tissue culture vessel in water, with concomitant problems of possible water leakage through the stopper.

Evidence has recently been offered that aureomycin in doses toxic to the tissue culture cells was successful in eliminating contamination by pleuropneumonia-like organisms[13]. This technique entails the use of an antibiotic not normally used in tissue culture, and the material must be added at each media change every 2 or 3 days. Additionally, it is known that there is a great variability in the toxic levels of aureomycin for any particular tissue culture cell line[13]. This would necessitate experimentation with each tissue culture cell line to determine the toxic dose. The hyperthermic technique obviates this disadvantage.

The advantages of the hyperthermic technique are: (1) no new chemical or antibiotic is introduced into the culture; (2) there is assurance that the pleuropneumonia-like organisms are killed, not merely arrested in growth by the bacteriostatic effect of antibiotics[11]; (3) intracellular pleuropneumonia-like organisms are also killed; (4) the method is very simple and easy to carry out.

This work was supported by a grant (*C*-4534) from the National Institutes of Health, United States Public Health Service.

Leonard Hayflick

Wistar Institute of Anatomy and Biology,
36th and Spruce Streets,
Philadelphia 4, Pa.

[1] Robinson, L. R., Wichelhausen, R. H., and Roizman, B., *Science*, **124**, 1147 (1956).
[2] Collier, L. H., *Nature*, **180**, 757 (1957).
[3] Rothblatt, G. O., and Morton, H. E., *Bact. Proc.*, **73** (1958).
[4] Hayflick, L., and Stinebring, W. R., paper presented at a Conference on the Biology of the Pleuropneumonia-like Organisms, *Ann. N.Y. Acad. Sci.*, **79**, 433 (1960).
[5] Hayflick, L., and Stinebring, W. R., *Anat. Rec.*, **121**, 477 (1955).
[6] Hayflick, L., Stinebring, W. R., Breckenridge, F. C., and Pomerat, C. M., *Bact. Proc.*, **75** (1956).
[7] Shepard, M. C., *J. Bact.*, **75**, 351 (1958).
[8] Leberman, P. R., Smith, P. F., and Morton, H. E., *J. Urol.*, **64**, 167 (1950).
[9] Leberman, P. R., Smith, P. F., and Morton, H. E., *J. Urol.*, **69**, 399 (1952).
[10] Tulasne, R., *Revue d'Immunologie*, **15**, 223 (1951).
[11] Edward, D. G. ff., *J. Gen. Microbiol.*, **10**, 27 (1954).
[12] Selawry, O. S., Goldstein, M. N., and McCormick, T., *Cancer Res.*, **17**, 785 (1957).
[13] Hearn, jun., J. H., Officer, J. E., Elsner, V., and Brown, A., *J. Bact.*, **78**, 574 (1959).

PATHOLOGY

Cerebellar and Midbrain Lesions in Scrapie

ATAXIA, inco-ordination of movement and to a limited extent disturbances in muscular tonus appear to be the main neurological symptoms in scrapie disease of sheep. Such symptoms suggested cerebellar lesions and indeed lesions have been demonstrated in the cerebellum[1-3], but only occasionally. Recently, an alteration has been made in our technique of taking material from the cerebellum for the routine histological examinations. Dorso-ventral sections have been taken through the middle of the cerebellum, parallel to the long axis of the brain, instead of the usual slice from a sagittal section through the vermis. Examination of such sections from nine brains of sheep affected with natural scrapie showed in each case degeneration and vacuolation of the Purkinje cells in a number of the folia of the paraflocculus (Fig. 1), while occasionally the neurones of the dentate nucleus were also affected. In the midbrains of these animals, in addition to vacuolation of the pigmented cells of the raphe, chromatolysis of the neurones of the red nucleus was a constant finding, while vacuolation was also often present. In the pons the neurones of the nucleus of the abducens nerve were always degenerated and vacuolated, but in the cells of the reticular formation changes were present in seven cases out of nine. In the medulla, lesions were found in the nuclei of the raphe, arcuate and formatio reticularis alba. Lesions of the above nuclei of the medulla have previously been shown to be constantly present in both natural and experimental scrapie[3,4].

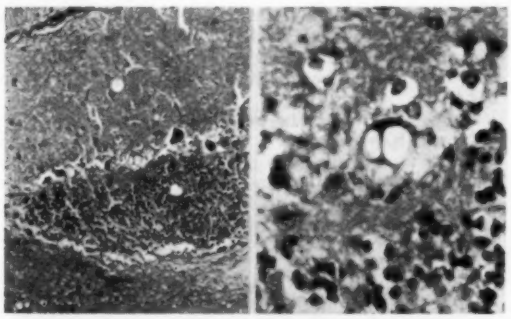


Fig. 1. Left, degeneration and vacuolation of the Purkinje cells (× 90); right, high-power magnification of a vacuolated Purkinje cell (× 360)

In the experimentally induced disease, although the clinical symptoms are identical with those of natural scrapie, lesions in the medulla and pons are not always very obvious[4] and although the distribution of such lesions as there are is similar to that seen in the naturally occurring disease the neurones undergoing degeneration are occasionally so few in number that it is difficult to relate the symptoms to the lesions. In the majority of experimental scrapie cases so far examined the neurones of the red nucleus of the mesencephalon were often affected as also were cells in the medulla and pons, but on occasion no lesions could be found in the midbrain even when 18 consecutive serial sections were examined. Recently, however, transverse sections of the mesencephalon from three such brains have been examined by cutting serial sections along the whole of the red nucleus. A total of 1,000 sections 7μ thick were cut but only every fourth section was examined, and in each of the three midbrains a varying number of the large neurones of the red nucleus was found undergoing either chromatolysis, pyknosis or necrosis. It would seem, therefore, that in all cases of scrapie the neurones of the red nucleus are affected. It is known that in lower mammals including the sheep the red nucleus serves as a centre through which the cerebellum can influence the motor functions of the spinal cord and medulla oblongata[5]. Lesions in the red nucleus and cerebellum will therefore cause lack of co-ordination of muscular activity as well as disturbances in tonus and synergy.

I. ZLOTNIK

Moredun Institute,
Gilmerton, Edinburgh. Nov. 11.

[1] Bertrand, I., Carré, H., and Lucam, F., *Rec. Méd. vét.*, **113**, 586 (1937).
[2] Wagner, A. R., Goldstein, H. E., Doran, J. E., and Hay, J. R., *J. Amer. Vet. Med. Assoc.*, **124**, 136 (1954).
[3] Zlotnik, I., *J. Comp. Path.*, **68**, 148 (1958).
[4] Zlotnik, I., *J. Comp. Path.*, **68**, 428 (1958).
[5] Ranson, S. W., "The Anatomy of the Nervous System" (W. B. Saunders Co., 1953).

Effect of Injection of a Hepatotoxin directly into the Gall-Bladder of Rabbits

AN inflammatory condition of the bile ducts is generally recognized to be the essential feature of 'facial eczema' liver damage in sheep[1]. This condition has recently been shown to be caused by a toxic principle elaborated by the fungus *Sporidesmium bakeri* Syd., a common species inhabiting many pastures in New Zealand (ref. 2 and Percival, J. C., unpublished work). Guinea pigs and rabbits have also been shown to be susceptible to the disease, liver damage of a generally similar nature having been produced by feeding toxic grass and crude fungal culture, and ether-extracts of these (Clare, N. T., unpublished work), and also by injection of extracts (Worker, N. A., unpublished work).

Recently, in a series of experiments on rabbits, in which the relative effectiveness of various routes of administration was compared, ether extracts of fungus, dispersed in 5 per cent aqueous 'Tween 40', were injected into: (1) the duodenum, proximal to the sphincter of Oddi; (2) the portal vein; (3) the gall-bladder.

Twenty New Zealand white rabbits of mixed sex, weighing 1,000–1,500 gm., were allotted to each group at random. Each rabbit was dosed with the equivalent of 1 litre of fungal culture dispersed in 0·4 ml. 'Tween'. The animals were killed at periods varying from four to seven days. Examination of the morbid anatomy and histology of the livers was carried out by D. C. Dodd, veterinary diagnostic officer of this Station.

From the results summarized in Table 1, it can be seen that after injection of extracts into the duodenum and into the portal vein, the majority of animals in both treatment groups exhibited mild to moderate liver damage, the lesions being similar both macroscopically and histologically to those commonly found after administration of toxic material by mouth (Dodd, D. C., unpublished work). In contrast to these results, however, injection of toxic extract into the gall-bladder produced, in every animal, lesions of the most severe form. The end result of this direct injection procedure was inevitably functional obliteration of the gall-bladder, with very severe generalized

Table 1. Gradings of Liver Lesions in Rabbits after Administration of Facial Eczema Toxin by Different Routes
Gradings based on macroscopic and histological examination

Route of administration	Grading			
	Mild	Moderate	Severe	Very severe
(1) Duodenum	7	11	2	0
(2) Portal vein	6	10	4	0
(3) Gall-bladder	0	0	0	20

œdema and fibrosis of the intra- and extra-hepatic biliary systems, together with gross enlargement of the liver and marked discoloration. A most outstanding feature of the results was the completely generalized effects of the treatment throughout all lobes of the liver. Twelve of the twenty animals so treated exhibited clinical icterus, a condition which was not noted in either of the other experimental groups and which in the past has been seldom encountered in rabbits in the course of other studies relating to this disease.

The results of the above studies represent a somewhat perplexing situation. In particular, the completely generalized effect noted throughout the liver of every animal in group 3 is puzzling and is difficult to explain satisfactorily in terms of the accepted facts of liver physiology and function. Expulsion of the toxic dose by way of the common bile duct followed by its subsequent resorption through the intestine would appear to be ruled out as a major contributing factor in view of the finding that injection of equivalent amounts of toxin into the duodenum (treatment 1) or the portal vein (treatment 2) produced damage of very much lesser severity. Similarly, it would appear that absorption of the dose through the venous drainage of the gall-bladder is not a factor of any great significance since cystic venous blood discharges into the portal circulation immediately adjacent to the liver, a situation as represented in treatment 2.

One possible explanation for this effect might be a retrograde movement of toxin from the gall-bladder into the biliary tree. Although it is difficult to visualize a diffusion of toxin against a bile flow which has been shown in this laboratory, in animals of similar size, to be of the order of 6–10 ml. per hr., such a hypothesis would provide an adequate explanation for the extreme severity of the lesions and for the uniform distribution of damaged biliary epithelial tissue throughout the entire substance of the liver.

This hypothesis clearly requires further investigation, not only in view of its general interest, but more particularly because of its possible implications in the pathogenesis of liver damage in facial eczema. If it can be demonstrated that, after concentration and storage in the gall-bladder, materials can re-enter and move freely within the biliary tree, it would suggest that in animals grazing under field conditions the function of the gall-bladder and biliary apparatus may be an important factor in the development of liver lesions. Attempts are being made with the aid of radio-opaque tracers to study gall-bladder function in more detail and to follow more closely the course taken by materials in transit from the gall-bladder to the intestine.

N. A. Worker

Ruakura Animal Research Station,
Department of Agriculture,
Hamilton, New Zealand.

[1] Cunningham, I. J., Hopkirk, C. S. M., and Filmer, J. F., *N.Z. J. Sci. Tech.*, **24A**, 185 (1942).
[2] Thornton, R. H., and Percival, J. C., *Nature*, **183**, 63 (1959).

Effects of Dehydration on the Mitochondria of the Mouse Kidney

The effect of complete withdrawal of water on the animal organism has been studied very little from the point of view of morbid anatomy. The changes observed have been included in the signs of general cell damage such as cloudy swelling, for example.

Modern histochemical techniques[1,2] have made it possible to get information not only about intracellular oxidative mechanisms but also about the physical state of the mitochondria. Employment of the techniques in this way can be referred to as 'mitochondrial assay'[3]. It has been possible to localize cellular damage within a few hours in sites where lesions observable with the classical methods of histology develop only after some days or weeks. By this means it has been shown that swelling of the mitochondria of the kidney is a very early sign of pathological change in a number of conditions, such as magnesium depletion[4], starvation[5], and vitamin D overdosage[6].

The effect of complete dehydration on the mouse kidney has now been studied using histochemical reactions for succinic dehydrogenase and diphosphopyridine nucleotide diaphorase[7]. Mice were killed after 1, 2, 3 and 4 days complete deprivation of water.

With both dehydrogenase and diaphorase methods mitochondrial swelling could be observed after only one day of water deprivation. The intramitochondrial formazan deposits became larger in a limited number of tubular cells, distributed over the whole nephron. During subsequent days mitochondrial swelling increased progressively; in Fig. 1 is shown the reaction for diphosphopyridine nucleotide diaphorase after 4 days of water deprivation. When the animals were allowed to drink water after 4 days dehydration, the mitochondrial changes were rapidly reversed and the picture returned to normal, as shown in Fig. 2.

It is known that the laboratory animals can concentrate their urine to a much greater extent than

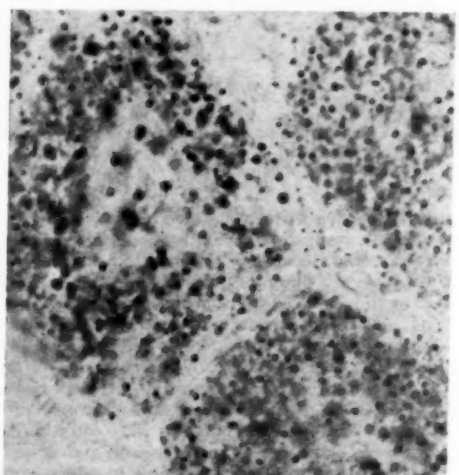


Fig. 1. Reduced diphosphopyridine nucleotide diaphorase reaction of the mouse kidney after four days complete water deprivation (× 1,800)

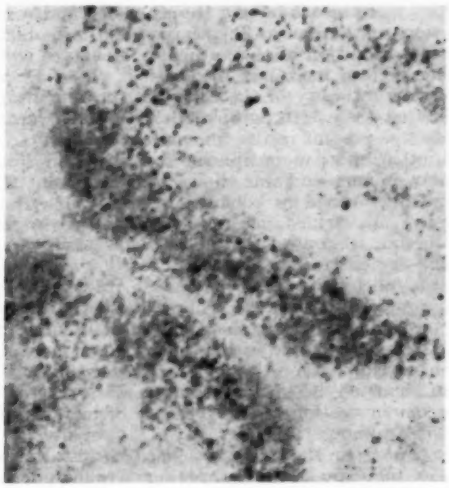


Fig. 2. Same reaction 24 hr. after allowing the dehydrated animal free access to water (× 1,800)

man. It has been believed that this ability to concentrate is a physiological function causing no morphological change in the cells. The results presented here suggest, however, that the high concentration of urine is achieved at the expense of a reversible mitochondrial damage.

Mikko Niemi*
N. Schor†
A. G. E. Pearse

Department of Pathology,
Postgraduate Medical School
of London, W.12.

* British Council Scholar.

† Scholar of the Asociacion Argentina para el progreso da las Ciencias.

[1] Pearse, A. G. E., and Scarpelli, D. G., *Nature*, **181**, 702 (1958).
[2] Hess, R., Scarpelli, D. G., and Pearse, A. G. E., *Nature*, **181**, 1531 (1958).
[3] Pearse, A. G. E., *J. Clin. Path.*, **11**, 520 (1958). "Histochemistry, Theoretical and Applied", second ed. (J. and A. Churchill, London, 1960).
[4] Hess, R., Macintyre, I., Alcock, N., and Pearse, A. G. E., *Brit. J. Exp. Path.*, **15**, 80 (1959).
[5] Scarpelli, D. G., *Exp. Cell. Res.* (in the press).
[6] Scarpelli, D. G., Tremblay, G., and Pearse, A. G. E., *Amer. J. Path.* (in the press).
[7] Scarpelli, D. G., Hess, R., and Pearse, A. G. E., *J. Biophys. Biochem. Cytol.*, **4**, 747 (1958).

A New Approach to Tumour Immunity

Only rarely and depending on special conditions has actively acquired immunity been demonstrated for tumours indigenous to and tested within inbred strains of mice[1–3] or rats[4]. For this reason doubts have been expressed as to whether 'tumour immunity' exists independent of isoantigenic differences. Experiments carried out in this laboratory and summarized in Table 1 have utilized inoculation into mice of inbred strains of tumours produced in F_1 hybrid mice derived from the corresponding parent strains. Mice of the parent strain in which the F_1 'hybrid tumour' failed to grow were challenged with a tumour indigenous to and specific for their own strain.

The parent strains were *C57BL*, *C3H*, and *AKR*, from which F_1 hybrids (reciprocal crosses) *C57BL* × *AKR* and *C57BL* × *C3H* were produced. Tumours induced in F_1 hybrids by subcutaneous injection of methylcholanthrene were serially transplanted into F_1 mice of the same origin. Tumour *CAF* in (*C57BL* × *AKR*) F_1 was an infiltrating fibrosarcoma, tumour *CCF* in (*C3H* × *C57BL*) F_1 a moderately well-differentiated mammary carcinoma; both tumours grew in 100 per cent of inoculated homologous F_1 mice. When inoculated into either of the parent strains, these hybrid tumours grew in some animals if sufficiently large numbers of tumour cells were given. The ability of F_1 tumours to grow in parent strains, in spite of immunogenetic differences, has been thoroughly analysed by Klein *et al.*[5]. We have as yet not investigated in detail factors affecting the transplantability of hybrid tumours *CCF* and *CAF* to mice of parent strains. After inoculation of hybrid tumours in increasing doses a percentage of mice of the three parent strains remained free of tumour growth. Upon challenge of *C57BL* mice with sarcoma-89, a fibrosarcoma indigenous to and specific for this strain[6], most of these animals proved resistant to this tumour, as shown in Table 1, while control *C57BL* mice inoculated at the same time succumbed to progressive tumour growth. No such immunity was observed when *C3H* and *AKR* mice, respectively, following inoculation of F_1 hybrid tumours, were afterwards subjected to challenge with tumour indigenous to their strain (transplantable *C3H* mammary carcinoma; *CAM* leukæmia of *AKR* strain): killing tumours developed in 100 per cent of these mice as they did in control mice.

At present no explanation can be offered for the immunizing ability of F_1 hybrid tumours for *C57BL* mice but not for *C3H* and *AKR* mice. Two possibilities may be considered: (1) specific immuno-

Table 1. Immunity to Strain-Specific Tumour of *C57BL* Mice inoculated with F_1 Tumour

Experiment No.	No. of mice		F_1 tumour inoculation*				No. of mice resistant to F_1 tumour		Sarcoma-89 inoculation*		No. of mice resistant to sarcoma-89	
	M	F	Type†	I	II	III	M	F	I	II	M	F
1	5	5	*CAF*	$2{\cdot}5 \times 10^5$	$3{\cdot}5 \times 10^5$ (8)	—	0	3	3×10^5 (6)	3×10^5 (9)	0	3
2	6	6	*CAF*	1×10^5	$3{\cdot}5 \times 10^5$ (4)	5×10^5 (3)	6	6	3×10^5 (4)	—	6	6
3	5	5	*CCF*	2×10^5	$5{\cdot}5 \times 10^5$ (3)	7×10^5 (3)	5	5	$3{\cdot}4 \times 10^5$ (4)	—	5	5
4	20	30	*CAF*	1×10^5	5×10^5 (3)	—	4	26	4×10^5 (5)	—	3	15

* Figures indicate number of viable (unstained) cells injected per mouse, with parenthetical numbers representing time interval (weeks) since preceding inoculation.

† For explanation, see text.

genetic mechanisms may be involved, such as presence of a specific antigen common to F_1 hybrid tumour and sarcoma-89. In this connexion, it should be noted that while the *CAF* tumour was derived from a methylcholanthrene-induced sarcoma as was also sarcoma-89, the *CAF* hybrid tumour originated from a mammary carcinoma of a host injected with methylcholanthrene. Study of possible immunizing ability present in normal tissues of F_1 has been initiated by injecting *C57BL* mice with splenic cells of (*C57BL* × *AKR*) F_1 mice prior to inoculation with sarcoma-89. (2) Another possibility is suggested by previous studies in which we found *C57BL* mice to be much more potent antibody producers than *C3H* and *AKR* mice, as judged from levels of antibodies after injection of heterologous red cells[7]. On this basis one might suspect that only *C57BL* mice, but not *AKR* and *C3H* mice, produced sufficient antibody to F_1 hybrid tumours.

This work is now being expanded, with inclusion of additional systems testing immunizing ability of F_1 tumours in other inbred strains in order to elucidate the phenomenon described.

Kurt Stern

Department of Pathology,
Chicago Medical School and Mount Sinai Hospital,
and Mount Sinai Medical Research Foundation,
Chicago 8,
Illinois.

[1] Foley, E. J., *Proc. Soc. Exp. Biol. and Med.*, **80**, 675 (1952).
[2] Prehn, R. T., and Main, J. M., *J. Nat. Cancer Inst.*, **18**, 769 (1957).
[3] Prince, J. E., Fardon, J. C., Nutini, L. G., and Sperti, G. S., *Cancer Res.*, **17**, 312 (1957).
[4] Goldner, H., Bogden, A. E., and Aptekman, P. M., *J. Immunol.*, **12**, 520 (1959).
[5] Klein, E., and Klein, G., *Transplant. Bull.*, **3**, 136 (1956).
[6] Dunham, L. J., and Stewart, H. L., *J. Nat. Cancer Inst.*, **13**, 1299 (1953).
[7] Davidsohn, I., and Stern, K., *J. Immunol.*, **72**, 216 (1954).

Presence of 3-Methoxy-4-hydroxyphenylglycol and Metanephrine in Phæochromocytoma Tissue

The ethereal sulphate of 3-methoxy-4-hydroxyphenylglycol, a metabolite of epinephrine and norepinephrine, has recently been demonstrated in the urine of normal individuals and was noted to be markedly increased in the urine of patients having phæochromocytoma[1]. The free compound has not been previously demonstrated and the possibility that conjugated (nor)metanephrine gives rise to this compound could not be ruled out. We have now been able to demonstrate the presence of free 3-methoxy-4-hydroxyphenylglycol in phæochromocytoma tissue from four different patients, as well as metanephrine in three of these tumours.

About 5 gm. of phæochromocytoma tissue was homogenized with 2 vol. of 0·5 *M* acetate buffer at *p*H 3, saturated with sodium chloride and extracted into 10 vol. of ethyl acetate. The ethyl acetate extract was then washed with salt-saturated acetate buffer at *p*H 7 and evaporated to dryness *in vacuo*. The residue was taken up in 10 ml. acetone, transferred to a 15-ml. centrifuge tube, and the solvent evaporated in a current of nitrogen until the volume was about 4 c.c. After centrifugation, 0·5 ml. of the supernatant fluid was applied to Whatman No. 1 paper and subjected to two-dimensional chromatography using *iso*propanol/5 per cent ammonia (8 : 2) and *n*-butanol/acetic acid/water (8 : 8 : 2) as the solvent systems. A spot was present having the same R_F and colour reactions as authentic 3-methoxy-4-hydroxyphenylglycol[1]. Addition of the authentic substance to the chromatogram increased the intensity of this spot. From the intensity of the colour of the spot, it was estimated that 5–10 μgm. of the glycol per gm. of tissue was present in the tumours.

Another spot, metanephrine[2], was identified in a similar manner and was shown to be different from normetanephrine by separation during chromatography. This substance was demonstrated in three of the four tumours.

The demonstration of unconjugated 3-methoxy-4-hydroxyphenylglycol of biological origin has not been previously reported. This supports the pathway for formation of the sulphate of this metabolite from the free compound, rather than from (nor)metanephrine sulphate. Although normetanephrine has been reported in phæochromocytoma[3], this is the first demonstration of the presence of metanephrine in these tumours.

We wish to thank Dr. Richard Crout, National Heart Institute, and Dr. Arthur Grollmann, jun., Johns Hopkins Hospital, Department of Medicine, for making available samples of phæochromocytoma tissue.

Irwin J. Kopin
Julius Axelrod

Laboratory of Clinical Science,
National Institute of Mental Health,
National Institutes of Health,
Bethesda, Maryland.

[1] Axelrod, J., Kopin, I. J., and Mann, J. D., *Biochim. Biophys. Acta* (in the press).
[2] Axelrod, J., Senoh, S., and Witkop, B., *J. Biol. Chem.*, **233**, 697 (1958).
[3] Sjoerdsma, A., *et al.*, *Science*, **127**, 876 (1958).

A Soluble Protective Antigen of *Trypanosoma brucei*

Trypanosoma brucei in quantities sufficient for antigenic analysis was obtained by harvesting the blood of rats on the third day of infection by the protozoon, when the fulminating parasitæmia is at its height. The trypanosomes were separated from the blood by centrifugation and the resulting mash was preserved by freeze drying. Several batches made in this way were pooled.

Rabbits inoculated with this material produced not only agglutinins for live trypanosomes but also antibodies precipitating with rat serum. These precipitins, a response to traces of serum proteins not removed during the separation of the trypanosomes from the blood, were removed by absorption with normal rat serum. The absorbed antiserum, no longer reacting with normal rat serum, nevertheless precipitated with some component in the serum of infected rats, from which the trypanosomes had been removed by filtration; suggesting that infected rat serum contained an antigen peculiar to the infected state. This antigen was designated the 'exoantigen' of *T. brucei*.

Antisera to the exoantigen, free of antibodies to normal rat serum, were prepared by inoculating normal rats with trypanosome-free infected rat serum. These antisera not only precipitated exoantigens, but also agglutinated living trypanosomes *in vitro*, suggesting strongly that the exoantigens were liberated from trypanosomes and were not of rat origin. About a million living trypanosomes, which had been

washed as thoroughly as possible, were injected into rabbits and in two or three weeks the protozoa proliferated sufficiently to produce the typical chronic infection, and to induce antibodies, which agglutinated trypanosomes and precipitated the exoantigen present in infected rat serum. It is evident from quantitative considerations that the precipitins were not induced by an abnormal rat metabolite carried on the original inoculum but by antigens liberated from the multiplying trypanosomes. First, the amount of rat serum proteins contaminating the inoculum was insufficient to stimulate antibodies to normal serum proteins and therefore to the certainly much smaller amounts of exoantigen present. Secondly, the million trypanosomes injected could not have been carrying an antigenically effective amount of an abnormal rat metabolite because grams of wet weight of dead trypanosomes from the rat are needed to induce a similar antibody response in rabbits.

The *in vitro* agglutination of living trypanosomes both by rabbit antisera to living trypanosomes and by rabbit or rat antisera to exoantigen was specifically inhibited by exoantigen. In gel-diffusion precipitin systems, the exoantigen formed a continuous line when opposed to adjacent reservoirs of each of the two kinds of antisera. Thus, antibody of the same specificity was induced whether the provoking antigen was living and multiplying trypanosomes or exoantigens in infected rat serum free of trypanosomes, leaving no doubt that the exoantigen was liberated from the trypanosomes and was not an abnormal rat metabolite. In an immuno-electrophoretic analysis of infected rat serum exoantigen appears as a slow-moving component, moving even more slowly than the serum γ-globulin.

Certain biological effects of free exoantigen indicate that it is concerned with the process of infection. When trypanosomes are collected from infected animals their viability *in vitro* largely depends on the type of medium in which they are contained. Washed suspensions of living trypanosomes were re-suspended in (1) Alsever's solution, (2) Alsever's solution plus normal rat serum, and (3) Alsever's solution plus infected rat serum containing exoantigen; they were injected into mice after standing at room temperature for 1–2 hr. Suspensions in Alsever's solution alone were usually non-infective. The time to death after injection of serum-treated preparations depended not only on the dose of trypanosomes, but also on the nature and amount of the added serum. Mice receiving trypanosomes and infected rat serum invariably died first, and the time to death was directly proportional to the concentration of exoantigen added. Mice receiving trypanosomes and normal rat serum always died later, if at all. Moreover, when suspensions of trypanosomes in normal and infected rat serum were held for varying periods on the bench, the older suspensions were the less infective, although the suspensions containing exoantigen remained infective for longer periods than those with normal rat serum. Since exoantigen appears to be relatively stable, it is unlikely to have been acting as an aggressin the potency of which was declining during the period on the bench; so that the increased infectivity of suspensions containing exoantigen appears mainly to be due to the more efficient preservation of the viability of the trypanosomes *in vitro*.

Nevertheless, though the exoantigen does not in these circumstances behave as a soluble aggressin, it has immunogenic properties. Repeated injection of exoantigen into mice and rats actively immunizes the animals against infection by live trypanosomes. Presumably, therefore, antibody to exoantigen is protective.

In conclusion, the exoantigen of *T. brucei* which occurs in the serum of infected rats plays an essential part in infection and is a means by which laboratory animals can be immunized against *T. brucei* by syringe inoculation. Its immunogenic efficiency against the infection transmitted by the tsetse fly—an experimental system closer to the natural disease—is under investigation.

B. G. F. Weitz

Lister Institute of Preventive Medicine,
Elstree, Herts.

Runt Disease and Tolerance in Rabbits

The production of immunological tolerance, that is, specific inhibition of the immune response in adult life by contact with antigen at an early age, was first described by Billingham, Brent and Medawar[1] and Hašek[2]. By this means, animals of different strains or breeds can be made to accept skin homografts from each other in adult life, provided they have been injected in the fœtal or early neonatal period with cells taken from their future skin donors.

The time in early life (adaptive period) during which the animal reacts to the administration of foreign cells by becoming tolerant varies considerably from species to species. In rats[3] it ends some days after birth, in mice at birth[4], but in rabbits incomplete evidence suggests it probably ends some time before the end of intra-uterine existence[5]. Moreover, when the cells used to induce this tolerance are adult and capable of forming antibody, a considerable proportion of the recipients may develop runt disease, characterized by chronic wasting and premature death[4].

Recently, as part of a study of graft-against-host reactions in the rabbit, it became necessary to produce runts in this species. As this has not been described before, and as detailed information on the adaptive period in rabbits was incidentally obtained, the results may be of interest to others.

Homologous spleen cells from adult female chinchilla rabbits were injected into newborn or fœtal rabbits. The donor animal was anæsthetized and splenectomy performed. The spleen was finely chopped in heparinized saline, and then suspended by gently drawing up and down through a wide-bore cannula. After filtration through nylon bolting cloth of 90-μ porosity, a cell count was made and a suitable quantity of the spleen suspension injected into the recipient. Newborn rabbits were injected intravenously within 1–4 hr. of birth. To inject fœtuses, a laparotomy was performed on a pregnant rabbit and the uterus delivered through the wound. The head and back of each fœtus were identified, and then spleen suspension injected into the peritoneal cavity of each. The uterus was returned to the abdomen and the wound sutured. When possible, the recipients of spleen cell suspension were challenged at 8 weeks with skin grafts from the spleen donor, skin homografts from another unrelated donor and autografts. These were full-thickness grafts 2 cm. in diameter sutured into prepared beds upon the ears[6].

Table 1. Incidence of Runt Disease and Survival Time of Skin Homografts in Rabbits given Homologous Spleen Cells in Fœtal Life or at Birth

Group	No. of rabbits injected	Route of injection	Age at time of injection	No. of dead at birth	No. of runts	Survival times of skin grafts from spleen donors (days)
25×10^6 cells injected						
I	8	Intravenously	1–4 hr. after birth	0	0	6, 6, 6, 6, 7, 7, 8, 9
II	10	Intraperitoneally	28th day in utero	1	0	6, 6, 6, 7, 7, 8, 8, 8, 9
III	9	,,	26th day in utero	1	0	6, 6, 7, 7, 7, 8, 8, 9
IV	10	,,	24th day in utero	1	0	9, 9, 9, 9, 10, 10, 11, 11, 12
V	12	,,	22nd day in utero	2	1	7, 8, 9, 12, 18*, 20, 24, 28, 34, 180
VI	12	,,	20th day in utero	5	3	14*, 24*, 36, 40, 80, 180
50×10^6 cells injected						
VII	18	,,	22nd day in utero	7	5	7, 8, 16*, 24, 38, 180
VIII	21	,,	20th day in utero	9	8	14*, 68, 90, 180, 180

* Runts with viable skin grafts at time of death of host.

It can be seen from Table 1 that the adaptive period in the rabbit ends about the twenty-second day of gestation (normally pregnancy lasts 31 days). Giving rabbits 25 ($\times 10^6$) spleen cells intravenously immediately after birth or intraperitoneally any time after the twenty-second day of fœtal life failed to produce immunological tolerance or runt disease.

Indeed, in fœtuses injected at the twenty-sixth day of pregnancy or later, skin homografts from the spleen donor were rejected more rapidly than corresponding homografts from unrelated rabbits, suggesting that immunity had resulted and that these pieces of skin were behaving as 'second-set' grafts[7]. In the 25 animals from groups I, II and III that were skin grafted, the mean survival times for the 'spleen donor' and 'unrelated' skin transplants were found to be $7{\cdot}12 \pm 1{\cdot}05$ and $10{\cdot}04 \pm 1{\cdot}21$ days, respectively, a difference which is significant ($P < 0{\cdot}01$).

After the critical twenty-second day there seems to be a brief 'neutral' period in which foreign cells are unable to induce either tolerance or immunity. Thus, in animals exposed to spleen cells at the twenty-fourth day of fœtal life (Group IV), there is no significant difference ($P > 0{\cdot}10$) between the survival times of skin homografts from the spleen donors and those of grafts from unrelated donors.

Of the 12 fœtal rabbits injected at 22 days (Group V), 6 became tolerant and showed definite prolongation of survival of skin homografts from the spleen donor, but not from the unrelated donor. Tolerance was not complete, and only one animal has an intact graft at the time of reporting (180 days). In another of the animals runt disease was encountered, as was only to be expected after the introduction of adult immunologically competent cells into an immature animal, and one of the two animals dead at birth showed the lymphoid atrophy usually associated with this syndrome.

In Group VI (injected at 20 days) all the surviving animals showed tolerance of varying degrees to spleen donor skin grafts. Runt disease was more common than in Group V and three of the five animals born dead showed evidence of the same process.

Increasing the dose of spleen suspension injected into 22- and 20-day old fœtuses to 50 ($\times 10^6$) nucleated cells greatly raised the incidence of runt disease and the number of rabbits born dead (Groups VII and VIII). 9 of the 16 animals dead at birth showed lymphoid atrophy. The degree of tolerance was greater and runt disease more frequent and severe among those treated at the twentieth day of fœtal life than with those treated at the twenty-second day. The animals which developed a high degree of tolerance without showing runt disease clinically gained weight more slowly than their less tolerant litter mates and showed some lymphoid damage.

Some of the rabbits in which runt disease was induced developed an immune hæmolytic anæmia. Investigations into this phenomenon and the histological changes found in association with the runting syndrome will be reported in detail elsewhere.

My thanks are due to Miss J. Rendall for technical assistance. This work was supported by a grant from the Medical Research Council.

K. A. Porter

Department of Pathology,
St. Mary's Hospital,
London, W.2.
Nov. 9.

[1] Billingham, R. E., Brent, L., and Medawar, P. B., *Nature*, **172**, 603 (1953).
[2] Hašek, M., *Csl. Biol.*, **2**, 25 (1953).
[3] Woodruff, M. F. A., and Simpson, L. O., *Brit. J. Exp. Path.*, **36**, 494 (1955).
[4] Billingham, R. E., and Brent, L., *Transpl. Bull.*, **4**, 67 (1957).
[5] Billingham, R. E., Brent, L., and Medawar, P. B., *Phil. Trans.*, B, **239**, 357 (1956).
[6] Stark, R. B., Brownlee, H., and Grunwald, R. P., *Ann. N.Y. Acad. Sci.*, **73**, 772 (1958).
[7] Medawar, P. B., *J. Anat.*, **78**, 176 (1944).

VIROLOGY

Electron-dense Crystallites in Nuclei of Human Amnion Cells infected with Measles Virus

Measles virus antigen has been specifically identified in the nuclei of infected cells by means of the fluorescent antibody technique; and the development and spread of virus antigen in amnion and other human cells have been correlated with observations made in a concurrent study of fine structure[1]. During a continuation of the latter investigation we have found that crystallites made up of ordered arrays of electron-dense particles occur within nuclei of infected human amnion cells, and have also observed extracellular particles that can be interpreted as mature virus.

Twenty-one days after the cells were infected with the Edmonston strain of measles virus, they were fixed in osmium tetroxide, and ultra-thin sections were prepared for electron microscopy. Sections were mounted on 200-mesh grids and floated for 15 min. on a saturated solution of uranyl acetate to enhance contrast.

Many of the cells contained intracytoplasmic inclusion bodies that have been described in detail[1]. In addition, the nuclei of a few cells contained

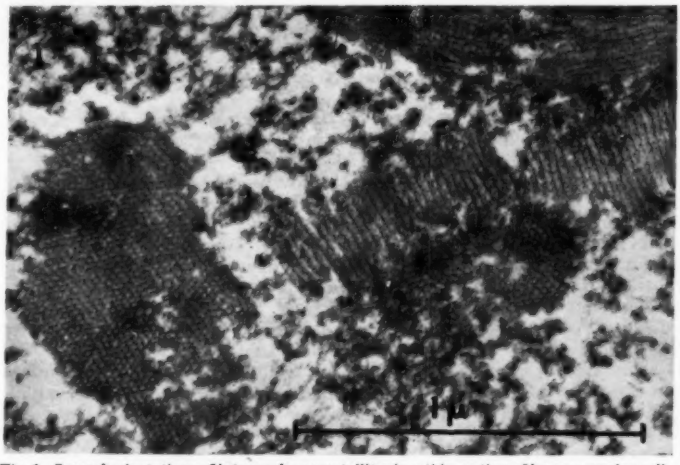


Fig. 1. Several orientations of intranuclear crystallites in a thin section of human amnion cell 21 days after infection with measles virus

crystalline arrays (Fig. 1). Crystalline arrays were not seen in uninfected amnion cells. It is reasonable to suppose that the arrays are associated in some way with virus multiplication. Three crystallographic directions can be identified in the crystallite at the left of Fig. 1, suggesting that a (111) plane is being visualized. The observed spacings along the three directions, in conjunction with the angles enclosed (70°, 70°, 40°), can be interpreted in terms of a unit cell with sides 120 Å. × 120 Å. × 211 Å. Other orientations are displayed by the crystallites at the right of Fig. 1, the spacings of which also are in agreement with the above interpretation.

It is unlikely that the crystallites consist of mature measles virus particles. Many of the cells containing crystalline arrays, or characteristic cytoplasmic inclusion bodies[1], or both, show particles on or outside the cell wall that might be interpreted as mature virus because of their morphology and size (Fig. 2). They exhibit double membranes, separated by 50 Å.; display an internal granular structure; and have an approximate diameter of 1200 Å. Benyesh *et al.*[2] have reported that the diameter of mature measles virus is approximately 1400 Å. Many of the bodies are 5- or 6-sided in outline, suggesting a polyhedral shape (Figs. 2 and 3).

Intranuclear crystallites have been observed in electron micrographs of animal cells infected with insect viruses and several mammalian viruses, including adenovirus[3] and herpes virus[4]. In some of the former and both the latter infections, the crystals appeared to be made up of the virus particles themselves. In addition, one type of crystal found in a type 5 adenovirus infection of HeLa cells was shown to be protein[4]. Morgan *et al.*[4] have discussed conditions that may favour the development of intracellular crystals in cells infected with virus, and speculate that the time-rate of virus assembly per unit volume may be the chief factor determining whether an ordered array will form.

Two mature animal viruses having polyhedral shapes have been demonstrated in electron micrographs. *Tipula* iridescent virus is 6-sided in cross-section and is icosahedral[5]. Adenovirus type 5 is also an icosahedron[6]. Three-dimensional studies are needed to determine which polyhedral shape might be assumed by mature measles virus.

This investigation was carried out under the sponsorship of the Commission on Viral Infections, Armed Forces Epidemiological Board, and was supported in part by the Office of the Surgeon General, Department of the Army, and in part by Grant *E*-2418, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Richard F. Baker
Irving Gordon
Fred Rapp*

Department of Medical Microbiology,
University of Southern California School of Medicine,
and
Los Angeles County General Hospital,
Los Angeles 33, California.
July 28.

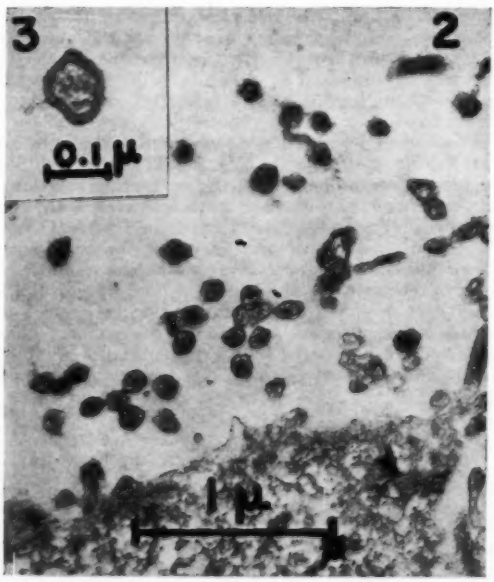


Fig. 2. Virus-like particles outside human amnion cell, showing internal structure and external membrane

Fig. 3. Higher magnification of a particle showing double membrane and suggesting polyhedral shape

* Present address: The Hospital for Special Surgery, Philip D. Wilson Research Foundation, 535 East 70th Street, New York.

[1] Rapp, F., Gordon, I., and Baker, R. F. (submitted for publication). Baker, R. F., Rapp, F., and Gordon, I. (submitted for publication).

[2] Benyesh, W., Pollard, E. C., Opton, E. M., Black, F. L., Bellamy, W. D., and Melnick, J. L., *Virology*, **5**, 256 (1958).

[3] Harford, C. G., Hamlin, A., and Parker, E., *Trans. Assoc. Amer. Phys.*, **68**, 82 (1955). Morgan, C., Howe, C., Rose, H. M., and Moore, D. H., *J. Biophys. and Biochem. Cytol.*, **2**, 351 (1956).

[4] Morgan, C., Godman, G. C., Howe, C., and Rose, H. M., *Trans. Assoc. Amer. Phys.*, **81**, 281 (1958).

[5] Williams, R. C., and Smith, K. M., *Biochim. Biophys. Acta*, **28**, 464 (1958).

[6] Valentine, R. C., and Hopper, P. K., *Nature*, **180**, 928 (1958). Horne, R. W., Brenner, S., Waterson, A. P., and Wildy, P., *J. Molec. Biol.*, **1**, 84 (1959).

SOIL SCIENCE

Removal of Fixed Ammonium from Clay Minerals by Cation Exchange Resins

Ammonium in clay minerals that is not replaceable by K^+ has been defined as fixed NH_4^+. This fixed NH_4^+ is replaceable to some extent by Na^+ and H^+, but the amount that can be replaced depends upon the extraction method used. Recent work[1,2] has shown that small amounts of K^+ or NH_4^+ in the extracting solution block the replacement of fixed NH_4^+. Thus, the efficiency of an equilibrium extraction of fixed NH_4^+ is decreased by the accumulation of displaced NH_4^+ in the extracting solution[2]. If a clay mineral containing fixed NH_4^+ were equilibrated with a cation exchange resin instead of a soluble salt, there would be less soluble K^+ or NH_4^+ in the system. A comparison of these extraction methods was made, therefore, to determine if there was also less blocking and thus more fixed NH_4^+ removed in a resin extraction.

Samples of Montana vermiculite (0·2 gm.), Wyoming bentonite (0·3 gm.) and Goose Lake Grundite (illite, 0·5 gm.) that had been NH_4^+-saturated and dried at 110°, 350° and 110° C., respectively, were equilibrated with Na^+-, K^+- and H^+-saturated samples of 'Amberlite *IR*-120' in 20 ml. of distilled water. In each case > 32 mesh resin material with an exchange capacity of 4·32 m.equiv. per gm. and a clay mineral : resin ratio of 1 : 20 (oven dry-weight basis) were used. Samples of the dried, NH_4^+-saturated clay minerals (0·2 gm. vermiculite, 0·3 gm. bentonite and 0·5 gm. illite) were also equilibrated with 20 ml. of 1 *N* sodium chloride and with 20 ml. of 1 *N* potassium chloride. The various systems were equilibrated at room temperature by shaking for periods of 2–8 days. The mixtures were then filtered, the residues were leached with 350 ml. of 1 *N* potassium chloride, and the NH_4^+ in the leachates was determined by distillation with potassium hydroxide. Before ammonium saturation, the illite was treated with a solution of sodium chloride and $NaB(C_6H_5)_4$ in order to remove K^+ and increase the NH_4^+ fixing capacity[3]. Illite samples that were not NH_4^+ saturated were included in each experiment, and the results obtained with the NH_4^+-saturated illite were corrected for nitrogen arising from that originally present in this material.

Table 1 gives the total NH_4^+ contents of the minerals (Kjeldahl analysis), their exchangeable NH_4^+ contents as determined by distilling 1-gm. samples with 400 ml. of 1 *N* potassium hydroxide, and the amounts of NH_4^+ removed by the resin and soluble salt extractions. In these extractions enough replacing cation was used to remove at least 80·6, 49·5 and 25·1 m.equiv. of NH_4^+ per 100 gm. from the vermiculite, bentonite and illite samples, respectively, if the distribution coefficient for the exchange of cations was 1.

Table 1. Amounts of NH_4^+ removed from NH_4^+-saturated Clay Minerals by Various Treatments (m.equiv. per 100 gm.)*

Treatment	Ammonium vermiculite	Ammonium bentonite	Ammonium illite
Kjeldahl digestion	81·4	50·4	25·3
Hydrogen resin	80·5	36·7	23·9
Sodium resin	80·5	36·3	23·2
Sodium chloride	66·5	33·0	22·4
Potassium resin	10·7	9·0	22·7
Potassium chloride	8·1	5·7	22·1
Potassium hydroxide distillation	2·8	24·6	22·6

* Mean of two or more determinations. The illite values are corrected for the NH_4^+ removed from similarly treated illite samples that were not NH_4^+-saturated.

Comparison of the results obtained by the resin and soluble salt extractions using the same replacing cation shows that although the resin method of extraction was the more effective with all three minerals, it was not markedly so except in the Na^+ treatment of vermiculite. When the NH_4^+ vermiculite was equilibrated with Na^+ (or H^+) resin essentially all of the NH_4^+ was removed, whereas only 76 per cent of the fixed NH_4^+ (that is, NH_4^+ not removed by distillation with potassium hydroxide) was removed by the sodium chloride treatment. It seems safe to assume that with this mineral the efficiency of the resin technique was due to a decrease in the blocking effect of the replaced NH_4^+, which limited extraction by the sodium chloride technique, since other experiments (to be reported later) indicate that it was not due to a selective adsorption of NH_4^+ by the resin or to a contact effect of the resin.

The influence of the resin on the removal of fixed NH_4^+ from illite and bentonite was very small, and essentially the same, whether Na^+ or K^+ was used. This suggests that with these minerals the effect of the resin was not due to a decrease in the blocking effect of displaced NH_4^+. More likely it was due to grinding of clay particles by the resin during the prolonged shaking period. This would also explain the observation that potassium resin extracted more NH_4^+ from vermiculite than did potassium chloride. In the vermiculite–resin extractions a distinct decrease in the size of the clay particles was observed.

The results in Table 1 show that the different treatments with K^+ (potassium hydroxide, potassium chloride, potassium resin) did not remove the same amount of NH_4^+ from the clay minerals. To define fixed ammonium, it is necessary, therefore, to specify the method of determination even when K^+ is used as the replacing cation. In the present work NH_4^+ that was not liberated as ammonia by distillation with potassium hydroxide was arbitrarily considered as being fixed.

Previous experiments with vermiculite have shown that the blocking effect of NH_4^+ or K^+ can be reduced by increasing the acidity of the extracting solution[2]. In the case of bentonite and illite, however, hydrogen resin was no more effective than sodium resin (Table 1). Also, instead of the increase observed with vermiculite, there was a decrease in the amount of NH_4^+ removed when the bentonite and illite samples were extracted with potassium chloride instead of potassium hydroxide. These results suggest that factors not encountered with vermiculite are involved in the fixation and release of NH_4^+ in bentonite and illite.

A. D. Scott
A. P. Edwards
J. M. Bremner

Agronomy Department,
Iowa State University,
Ames,
Iowa.

[1] Hanway, J. J., *et al.*, *Soil Sci. Soc. Amer. Proc.*, **21**, 29 (1957).
[2] Scott, A. D., *et al.*, *Soil Sci. Soc. Amer. Proc.*, **22**, 383 (1958).
[3] Hanway, J. J., *Iowa State Coll. J. Sci.*, **30**, 374 (1956).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, March 14

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Prof. E. Zaimis: "Neurochemical Pharmacology".*

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6 p.m.—Informal Meeting on "Should Engineers be encouraged to take up Administrative Positions early in their Careers?".

Tuesday, March 15

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 1.30 p.m.—Prof. C. [D. Darlington, F.R.S.: "Heredity and Human Society".*

BRITISH COMPUTER SOCIETY, LONDON BRANCH (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 2.30 p.m.—Mr. N. Doveton: "Axis Transformation using Analogue Equipment".

UNIVERSITY OF LONDON (at University College Hospital Medical School, University Street, London, W.C.1), at 4.30 p.m.—Prof. M. Maizels: "Salt and Water Balance in Cells".*

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Sir Alan Burns: "Recent Developments in Fiji".

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. I. C. Hutcheon and Mr. D. Summers: "Fast-Response Transistor Chopper-Type Amplifier with Low Carrier Frequency".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Prof. H. B. Squire, F.R.S.: "Some Problems of Separated and Vortex Flow".

Tuesday, March 15—Thursday, March 17

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Royal Horticultural Society's New Hall, Westminster, London, S.W.1)—Twelfth Technical Exhibition.

Wednesday, March 16

ROYAL SOCIETY OF ARTS (at John Adam Street, London, W.C.2), at 2.30 p.m.—Dr. D. Roy Davies: "Induced Mutation in Crop Plants—Prospects and Prospectives" (Fernhurst Lecture).

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. D. V. Ager: "The Epifauna of a Devonian Spiriferid"; Dr. G. Kelling: "The Stratigraphy and Structure of the Ordovician Rocks of the Rhinns of Galloway".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. D. W. Martin and Mr. A. W. Brewer: "A Synoptic Study of Day-to-Day Changes of Ozone over the British Isles"; Mr. E. B. Kraus: "Synoptic and Dynamic Aspects of Climatic Change".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. P. A. Stone: "Economics of Building Designs".

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. D. H. Grindell: "An Electrostatic Dust Monitor".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Miss D. M. Brasher: "The Mechanism of Inhibition in Neutral Aqueous Solutions".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the LONDON SECTION of the ROYAL INSTITUTE OF CHEMISTRY, at University College, Gower Street, London, W.C.1), at 6.30 p.m.—Prof. F. D. Richardson: "High Temperature Reactions Involving Condensed Phases".

SOCIETY FOR PSYCHICAL RESEARCH (at Kensington Town Hall, Kensington High Street, London, W.8), at 7 p.m.—Mr. H. Norman Hunt will reproduce recordings from Spiritualist Mediums.

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. A. H. Doveton and Mr. K. C. W. Pedder: "The Simulation of a Large Chemical Plant on an Electronic Analogue Computer".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 7.30 p.m.—Dr. A. L. Glenn: "Hydrogen Ions and the Pharmacist".

Thursday, March 17

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. A. R. Peacocke and Mr. B. N. Preston: "The Action of γ-Rays on Sodium Deoxyribonucleate in Solution. II: Degradation. III: Denaturation"; Mr. J. Seed: "Inhibition of Nucleic Acid Synthesis Caused by X-irradiation of the Nucleolus".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. V. H. Heywood: "Progress in the *Flora Europaea* Project"; Mr. Eric M. Marsden-Jones: "The Life Cycle of *Adleria kellari* Hartig (Cynipidae)".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. P. J. Higgins: "Two Methods in Combinational Analysis".

INSTITUTE OF PHYSICS, LONDON AND HOME COUNTIES BRANCH (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Prof. F. P. Bowden, C.B.E., F.R.S.: "The Friction and Deformation of Solids at Very High Speed".

ROYAL AERONAUTICAL SOCIETY (at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. M. B. Morgan: "Supersonic Aircraft—Promise and Problems.".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. P. H. Robin: "Floors Old and New".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at the Royal Army Medical College, Millbank, London, S.W.1), at 7.30 p.m.—Laboratory Meeting. Chairman: Sir William MacArthur, K.C.B., D.S.O., M.D., F.R.C.P.

BRITISH INSTITUTE OF RADIOLOGY, incorporated with the ROENTGEN SOCIETY (at 32 Welbeck Street, London, W.1), at 8 p.m.—Meeting on "The Reduction of Radiation Hazards in the Therapeutic Use of Small Multiple Radioactive Sources".

Friday, March 18

BRITISH PSYCHOLOGICAL SOCIETY, OCCUPATIONAL PSYCHOLOGY SECTION (in the Department of Psychology, Birkbeck College, Malet Street, London, W.C.1), at 1 p.m.—Dr. Marie Jahoda: "Some Remarks on the Study of Technological Education".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. J. A. Fraser Roberts: "Medical Genetics".*

ROYAL SOCIETY OF MEDICINE, EPIDEMIOLOGY AND PREVENTIVE MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Dr. W. R. S. Doll and Dr. W. M. Court Brown: "Studies on the Possible Effects of Low Doses of Radiation".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (joint meeting with the GRADUATE AND STUDENT SECTION of the INSTITUTIONS OF CIVIL and MECHANICAL ENGINEERS, at the Institution of Mechanical Engineers, Birdcage Walk, London, S.W.1), at 6.30 p.m.—Address by Sir George Edwards, C.B.E.

SOCIETY FOR ANALYTICAL CHEMISTRY (joint meeting with the FINE CHEMICAL GROUP of the SOCIETY OF CHEMICAL INDUSTRY, at the School of Pharmacy, Brunswick Square, London, W.C.1), at 6.30 p.m.—Meeting on "Techniques of Automatic Analysis". Mr. R. M. Pearson: "Automatic Analysis in the Chemical Industry"; Dr. I. D. P. Wootton: "Automation in Clinical Biochemistry".

ANTIQUARIAN HOROLOGICAL SOCIETY (at the Science Museum, South Kensington, London, S.W.7), at 7 p.m.—Discussion on the work of the Ellicotts.

BRITISH INSTITUTE OF RADIOLOGY, incorporated with the ROENTGEN SOCIETY (at 32 Welbeck Street, London, W.1), at 8 p.m.—Meeting on "Tritium Counting".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. V. Jones: "Infra-red Radiation".

Saturday, March 19

NUTRITION SOCIETY (at the Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, E.C.1), at 9.45 a.m.—Symposium on "Nutrition and the Elderly".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SENIOR LECTURER IN HYDRO- AND AERO-DYNAMICS; and a SENIOR LECTURER IN PHYSICS—The Registrar, College of Advanced Technology, Gosta Green, Birmingham 4 (March 19).

ASSISTANT LECTURER IN THE DEPARTMENT OF MATHEMATICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (March 22).

LECTURER IN APPLIED MATHEMATICS—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (March 26).

LECTURER (with medical or scientific qualifications) IN PHYSIOLOGY—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (March 26).

RESEARCH FELLOWS (Ph.D. standard, with a special aptitude for research in high or low energy nuclear physics) IN EXPERIMENTAL OR THEORETICAL NUCLEAR PHYSICS—The Registrar, The University, Liverpool (March 26).

LEVERHULME RESEARCH FELLOW (graduate in medicine or science)—The Secretary, St. Mary's Hospital Medical School, Paddington, London, W.2 (March 28).

RESEARCH FELLOW (with research experience in biochemistry or chemistry, and an interest in the physico-chemical aspects of biochemistry) IN THE DEPARTMENT OF PHYSICAL BIOCHEMISTRY, John Curtin School of Medical Research, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (March 30).

ASSISTANT EXPERIMENTAL OFFICER (preferably woman aged at least 22, with B.Sc. pass degree, with bacteriology as principal or subsidiary subject, or equivalent) AT TORRY RESEARCH STATION, D.S.I.R., Aberdeen, to work on the maintenance of type cultures for the National Collection of Industrial Bacteria—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting Ref. G.68/0A (March 31).

ASSISTANT LECTURER IN PHILOSOPHY, tenable in either the Department of Philosophy, or in the Department of Political and Moral Philosophy—The Registrar, University College of North Staffordshire, Keele, Staffs (March 31).

CHAIR OF PHYSICS at University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 31).

DEMONSTRATORS (2) (graduates) IN AGRICULTURE, for duties on the University farms—The Registrar, The University, Leeds 2 (March 31).

FOULERTON GIFT RESEARCH FELLOW (British nationality) for original research in Medicine—The Assistant Secretary, The Royal Society, Burlington House, Piccadilly, London, W.1 (March 31).

JUNIOR LECTURER IN BOTANY—The Registrar, Trinity College, Dublin (March 31).

LECTURER OR ASSISTANT LECTURER IN MECHANICAL ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (March 31).

LECTURER or DEMONSTRATOR IN AGRICULTURAL BIOCHEMISTRY—The Registrar, King's College (University of Durham), Newcastle upon Tyne 1 (March 31).

RESEARCH FELLOW IN THE BIOPHYSICS LABORATORY, Department of Physics, for work largely concerned with the electric properties of plant membranes and tissues and the relation of these to plant development—The Registrar, University of Tasmania, Box 252C, G.P.O., Hobart, Tasmania (March 31).

SENIOR LECTURER or LECTURER IN MATHEMATICS at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (March 31).

WHITE'S PROFESSOR OF MORAL PHILOSOPHY—The Registrar, University Registry, Oxford (March 31).

RESEARCH WORKER IN PSYCHOPHARMACOLOGY for work concerned with the effects of psychotropic drugs on psychological and physiological functions, and will involve studies on normal subjects and psychiatric patients—Dr. H. O. Schild, Department of Pharmacology, University College, Gower Street, London, W.C.1 (April 2).

CHAIR OF BIOCHEMISTRY—The Registrar, The University, Leicester (April 8).

LECTURER IN SOCIAL ANTHROPOLOGY; and a LECTURER IN AGRICULTURAL CHEMISTRY—The Secretary of University Court, The University, Glasgow (April 11).

LECTURER IN CHEMISTRY in the Department of Chemistry and Chemical Engineering, University of Natal, Durban—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, April 15).

LECTURER or ASSISTANT LECTURER IN ZOOLOGY at the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

LECTURER/SENIOR LECTURER (qualified in grassland management) IN ANIMAL HUSBANDRY at the University of Sydney Animal Husbandry Farms, Camden, New South Wales—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, April 15).

LECTURER (with research interests in either branch) IN INORGANIC and/or PHYSICAL CHEMISTRY; a LECTURER IN MATHEMATICS; and a LECTURER IN MORAL PHILOSOPHY in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews (April 16).

ASSISTANT LECTURER (with special qualifications in the genetics of micro-organisms) IN THE SUB-DEPARTMENT OF GENETICS—The Registrar, The University, Liverpool, quoting Ref. CV/T (April 19).

RESEARCH FELLOW IN PURE SCIENCE; a RESEARCH ASSISTANT (preferably with a special interest in educational psychology and/or general experimental psychology) IN PSYCHOLOGY; and a GRADUATE ASSISTANT IN ZOOLOGY, with special responsibility for the Departmental Museum—The Secretary, The University, 38 North Bailey, Durham (April 23).

SENIOR LECTURER (with a postgraduate degree in either geography or geology from a British university or the equivalent, and teaching experience at university level) IN GEOGRAPHY AND GEOLOGY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, April 30).

ENGLISH ELECTRIC RESEARCH FELLOW IN AERONAUTICS, for postgraduate research into the problems of vibration or stability and control of aircraft—The Warden, The College of Aeronautics, Cranfield, Bletchley, Bucks (May 1).

PROFESSOR OF ENGINEERING SCIENCE—The Registrar, University Registry, Oxford (May 5).

BIOCHEMIST (B.Sc. or preferably Ph.D., with experience in bacteriology)—The Secretary, Wright Fleming Institute, St. Mary's Hospital Medical School, Paddington, London, W.2.

BIOCHEMIST, Senior grade, to take charge at East Riding Group Laboratory—The Secretary, Westwood Hospital, Beverley, East Yorkshire.

EXPERIMENTAL OFFICERS/ASSISTANT EXPERIMENTAL OFFICERS (with G.C.E. "A" level in chemistry and another science or mathematics subject or equivalent, but if over 22, a pass degree, H.N.C. in chemistry or equivalent is generally expected) AT THE NATIONAL CHEMICAL LABORATORY, D.S.I.R., Teddington, Middx., for work on general inorganic chemistry, microanalysis, electro-chemistry, chemical thermodynamics and corrosion—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1 quoting Ref. F.123/0A.

HOSPITAL PHYSICIST TO THE RADIOTHERAPY DEPARTMENT—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

LABORATORY TECHNICIAN IN THE AGRICULTURAL BOTANY SECTION of the Department of Agricultural Sciences—The Secretary, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough.

LECTURER or ASSISTANT LECTURER (man or woman graduate, preferably with teaching experience) IN PHYSICS in the Training Department—The Warden, Goldsmiths' College (University of London), New Cross, London, S.E.14.

MATHEMATICIANS (with a university degree and experience in working with digital computers) with the European Organization for Nuclear Research—CERN, Geneva, for the analysis and programming of various computing problems, in particular problems of numerical analysis on a Ferranti Mercury and IBM 704—The Ministry of Labour (E.9), 26–28 King Street, London, S.W.1.

PHYSICAL CHEMISTS (with a good honours degree and preferably some postgraduate research experience), to carry out fundamental work on the reaction between metals and water at high temperatures—The Director, Arthur D. Little Research Institute, Inveresk, Midlothian, Scotland.

RESEARCH ASSISTANT (graduate, preferably with experience in microanalytical techniques and chromatography), for work on biosynthesis and identification of steroid hormones in the adrenal gland—Mr. P. J. Ayres, Barnato Joel Laboratories, The Middlesex Hospital, Mortimer Street, London, W.1.

RESEARCH ASSISTANT (honours B.Sc. with experience in blood grouping, biochemistry, bacteriology or mammalian genetics), for work on transplantation immunity, including mouse blood group genetics, C.F., skin transplantation—Dr. H. B. Amos, Roswell Park Memorial Institute, Buffalo 3, New York, U.S.A.

SCIENTIFIC OFFICER (graduate, preferably in physics, and capable of maintaining and developing the heart lung machine and electronic and other research apparatus) to assist in developments related to cardiac surgery—The Assistant Administrator, Radcliffe Infirmary, Oxford.

SENIOR SCIENTIFIC and SCIENTIFIC OFFICERS (with a first- or second-class honours degree or equivalent in science, engineering or mathematics), for a very wide range of research and development work, including solid state physics, radio astronomy, advanced computing techniques, and electronics and radar—The Director, Royal Radar Establishment, Malvern, Worcs.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Scientific and Industrial Research: Road Research Laboratory. Road Note No. 24: Roadstone Test Data presented in Tabular Form. Pp. 8. (London: H.M. Stationery Office, 1959.) 9*d*. net. [3012

Laboratory Animals Centre. Collected Papers, Vol. 8 (1959): Report of a Symposium on Quality in Laboratory Animals, held at the Royal Veterinary College, London, on 16th April, 1959. Pp. 68. (Carshalton: Laboratory Animals Centre, M.R.S. Laboratories, 1959.) 10*s*. [3012

Department of Scientific and Industrial Research. Food Investigation 1958: Reports of the Superintendents of the Low Temperature Research Station and the Ditton Laboratory. Pp. vi+42. (London: H.M. Stationery Office, 1959.) 3*s*. net. [3012

Society for the Promotion of Nature Reserves. Handbook 1959: Forty Second Annual Report, year ending 31st March, 1959. Pp. 56+4 plates. (London: The Society for the Promotion of Nature Reserves, c/o British Museum (Natural History), 1959.) 10*s*. [3012

Other Countries

Arbeitsgemeinschaft für Forschung des Landes Nordrhein-Westfalen Heft 78: Experimentelle Studien über die Distanzeffekte in Bestrahlten Vielzelligen Organischen. Von Fréderic Ludwig. Die Anwendung Radioaktiver Isotope in der Chemischen Forschung. Von A. H. W. Aten, Jr. Pp. 88. (Köln und Opladen: Westdeutscher Verlag, 1959.) 9.20 D.M. [3012

The Story of Chemical Industry in Basle. Pp. 234. (Published by CIBA Limited on the Occasion of its 75th Anniversary.) (Basle: CIBA Limited, 1959.) [3012

Chicago Natural History Museum. Fieldiana: Zoology. Vol. 39, No. 31 (May 22, 1959): Inland Mollusks from Venezuela, Southern Brazil, and Peru. By Fritz Haas. Pp. 363–371. (Chicago: Chicago Natural History Museum, 1959.) 30 cents. [3012

Profiles of the Time of James Monroe, 1758–1831: an Exhibition Commemorating the 200th Anniversary of the Birth of the Fifth President of the United States, October 26–November 23, 1958. Pp. 32. (Publication 4348.) (Washington, D.C.: Smithsonian Institution, 1959.) [3012

Smithsonian Miscellaneous Collections. Vol. 139, No. 5: Genera of Tertiary and Recent Rhynchonelloid Brachiopods. By G. Arthur Cooper. Pp. iii+90+22 plates. (Publication 4382.) (Washington, D.C.: Smithsonian Institution, 1959.) [3012

Mitteilungen der Prähistorischen Kommission der Österreichischen Akademie der Wissenschaften. VIII und IX Band: Willendorf in der Wachau: Monographie der Paläolith-Fundstellen I–VII. 1 Teil: Text. Pp. v+219. 2 Teil: Inventar. Pp. 79. 3 Teil: Abbildungen. Abb. 124. (Wien: Österreichische Akademie der Wissenschaften, 1959.) [3012

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.,
ST. MARTIN'S STREET, LONDON, W.C.2.
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £7/15/–, payable in advance, postage paid to any part of the world

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office